STUDIES IN GROWTH

I. SUFFOCATION EFFECTS IN THE CHICK EMBRYO

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FIFTEEN FIGURES

INTRODUCTION

While making a study of some garter-snake embryos, the author observed what he construed as hemogenesis accompanying the development of large sinuses in the penes. This material seemed to show a transformation of mesenchyme into erythrocytes. Since the embryos showed only a venous circulation through the penes during the stage at which the sections were made, it occurred to the author that possibly lack of oxygen or accumulation of the end products of metabolism might in some manner affect hemogenesis and vascular growth generally. This idea is not new, for it was found on investigation that H. E. Jordan had on several occasions suggested that hemogenesis might be occasioned by accumulation of CO_2. It was in the hope of testing this hypothesis by a method that, if the hypothesis were correct, must give much more conclusive evidence than studies of normal development that this study was undertaken.

LITERATURE

Relatively little work has been done on embryonic respiration. The earliest recorded observation of the need of air by the chick embryo is that of Home(7). He rather naively states that he placed an embryo in water and that it died from suffocation. About 1882, Gerlach and Koch(5) performed several series of experiments on incubating eggs. They

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coated eggs with varnish except for a spot 4 to 6 mm. in diameter. This spot was oriented so that it lay in various relations to the position of the embryo in different eggs. They obtained abnormal development by moving this 'Luftfleck' away from the embryo or normal development by placing it directly over the embryo. Paul Mitrophanow (20; '00) experimented with development of early-stage monsters by variation of temperature and gaseous medium. Paton(23; '11), McWhorter and Whipple('12), and Vogelaar and van den Boogert(27; '25), among others, have developed the chick embryo outside the shell with varying degrees of success.

Riddle(24; '24), worked out critical points for oxygen and carbon-dioxide pressures. He states that high oxygen pressures produce abnormal embryos almost without hemoglobin. The paper of Henry A. Murray, Jr.(22; '25), contains a table of normal oxygen intake and carbon-dioxide output throughout the incubation period for White Leghorn eggs under standard conditions. Koehler, Benell, Behneman, and Loevenhart(10; '25), working with the pig, ascribe death from anoxemia to an ultimate acidosis in most cases. There are other papers related to this general subject, but they are either irrelevant or unavailable. Only those actually read in the preparation of this article have been cited; the bibliographies of the papers cited, especially that of Vogelaar and van den Boogert, contain titles of others dealing with early experiments with varnished eggs. Literature related to the morphological and physiological aspects of this problem will be cited in its proper place.

METHODS AND MATERIALS

As material for determining the relationship between hemogenesis and respiratory exchange, the hen's egg seemed to combine the most favorable features for research. Fertilized eggs are relatively easy to obtain and the normal embryology of the chick is better known than that of any other species. Further, since each embryo carries with it its

own immediate environment, it should prove easier to eliminate extraneous factors, e.g., effects of individuals on one another, that might prove troublesome in the use of free-living embryos.

In attacking the problem, a method was sought which would be so simple that it could be carried out in any laboratory without special apparatus and still give results sufficiently striking to be convincing. After experimenting with shellac and paraffin coatings and submergence in water and glycerol, coating the egg with water-glass was tried. Early experiments were by no means convincing, as total preincubation exclusion of air caused death of the embryo in a short time and some of the other experiments failed to affect development at all. The first applications of water-glass were no better; the embryos developed normally for several days. Finally, the following method was adopted and gave a reasonable degree of success.

It was discovered that if the embryo was allowed to start development and to develop for about twenty-four hours, it would live much longer after the elimination of respiratory exchange. On a basis of this fact, eggs were allowed to develop for twenty-four hours, then the air sac at the large end of the egg was opened and the whole egg, including the open air sac, was coated with concentrated water-glass by immersing the egg in a solution of it, and returned to the incubator. That is, the shell and the outer shell membrane at the large end of the egg were broken by tapping with the handle of a scalpel and the fragments lying over the air sac were removed. The inner shell membrane was left intact. This procedure hastens suffocation. Results were sufficiently sharp to warrant the temporary adoption of the method.

However, this all took time and eggs; out of ten dozen eggs subjected to this technique, five dozen had to be discarded because the supposedly normal controls were abnormal. Fifteen eggs from the remaining five dozen were run as controls, twenty-four were either normal, died very soon after coating, or were infertile; two embryos were accidentally destroyed, and thus nineteen embryos remained for this study.

One sees at a glance that this method leaves much to be desired. Just previous to the time this paper was submitted for publication, the author placed three eggs in a 300-cc. glass jar before incubation and without treatment of any sort, corked the jar tightly and placed it in the incubator and left it there for five days. One of the embryos so obtained has been sectioned and stained; it confirms the results obtained by the use of water-glass very strikingly.

At present the author is working to standardize a third technique that should give more uniform results than treatment with water-glass; but absolute uniformity cannot be expected, for the viability of strictly fresh eggs from the same flock varies. This technique is as follows: an egg normally incubated for twenty-four hours is subjected to the removal of a portion of its shell so that the embryo is visible. The egg is then placed in an air-tight container of small volume, the container is tightly corked and returned to the incubator. The time of the cessation of heart beat is recorded as nearly as possible and the embryo is removed and fixed at a definite time afterward.

Before this technique can be applied, it must be demonstrated that such eggs will develop normally when supplied with air. 'Normal' determinations are now being run. So far the best results have been obtained by removing about a square inch of the shell and the accompanying shell membranes near the large end of the egg and placing the egg with the large end up in the neck of a wide-mouthed bottle just large enough to receive the smaller half of the egg, but not large enough to permit the egg to slip completely into the bottle. The bottle is then set in a pan and covered with a beaker just tall enough so that the bottom of the beaker clears the egg when the beaker is inverted over the bottle. This procedure cuts albumen loss to a minimum and reduces evaporation. The embryo orients itself so that it lies at the edge of the exposed area. Such embryos have developed normally to the seventh day of incubation. If the upper third of the shell is removed when the egg is in a horizontal posi-

tion, considerable albumen is lost and the embryo usually does not live beyond the end of the fourth day of incubation. Variations in this technique will prove very useful for class observations on the living chick in course work in embryology.

Serial sections of the embryos studied were cut from 3 to 7 μ in thickness and stained with haematoxylin and erythrosin or with eosin-azur after the technique of Maximow(16). Fixation for the haematoxylin and erythrosin sections was with Bouin's fluid and for eosin-azur with Helly's fluid.

RESULTS

All nineteen of the embryos from the eggs treated with water-glass had been allowed to incubate for seventy-two hours after the eggs were coated with water-glass, and were thus embryos of approximately ninety-six hours' incubation. They varied from embryos with a feebly developed circulatory system to embryos with no circulatory system and with great abnormality of form. Under gross examination, only the anterior portion of some of them seemed to approximate normal body form to any recognizable degree. Most of them contained large patches of blood within the body. The most remarkable feature of the group was the fact that none of them showed a well-developed allantois. In fact, before sectioning no allantois could be discoverd. On sectioning, all of the embryos were found to have extraordinarily large blood vessels and many anomalous sinuses. Some of the vessels contained large amounts of blood, while others were nearly empty. The blood contained a large percentage of young erythroblasts and old, hemolyzed erythrocytes.

A constantly recurring fatty necrosis was present in these embryos. It reached its height in the optic and cephalic vesicles and diminished posteriorly in the tube to degeneration of the floor plate alone or to a normal condition. The optic vesicle next the yolk uniformly showed greater degeneration than the superficial one. Similar necrosis was present to a greater or less degree in the mesodermic somites and the somatopleure. The fatty nature of the degenerative

changes was demonstrated by staining with osmic acid; the detritus would not stain with sudan III. Normally active cells were present contiguous to degenerating areas. This was especially striking in certain areas of the neural tube in which degeneration had but little exceeded the region of the floor plate. Mitotic figures were normally or even abnormally abundant in such areas.

These general results may be divided for closer examination into four major divisions: 1) the relation between allantoic development and respiration; 2) relation between hemogenesis and respiration; 3) relation between development of the circulatory system and respiration; 4) relation between cellular proliferation and respiration.

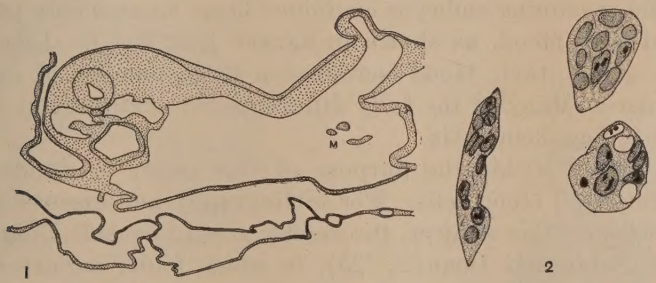
RELATION BETWEEN ALLANTOIC DEVELOPMENT AND RESPIRATION

The allantois functions in two ways in the embryo—as a repository for the liquid wastes of metabolism and as a respiratory area. In the embryos used in this study, the possibility of serving as a respiratory area was completely or almost completely removed. It cannot be supposed that absolutely no oxygen reached these embryos from the outside; but, under the experimental conditions, very little could have entered through the shell. The production of liquid wastes may by no means be considered as having stopped with reduction of intake of atmospheric oxygen. On the contrary, these wastes probably increased in amount. But circulation of blood did not go on in these embryos, even in such of them as had a complete circulatory system, with normal vigor. Thus liquid wastes tended to remain in the tissues rather than to be carried to the allantois.

The great sinuses formed by enlargement of the cardinal veins are undoubtedly at least partly formed in this way. As shown in figure 1, the mesonephric tubules remain as islands of fairly normal tissue at the edge of such sinuses. Apparently, they may sometimes be cut off entirely and exist free in the cavity of the sinus itself. The example shown in figures 1 and 2 was followed carefully from section to

section and no peripheral connection could be found. The example shown is an extreme case. The relationship is commonly that shown in figure 10, in which specimen a mesonephric tubule of the right side is shown supported in a thin trabecula of mesenchyme.

According to the work of Fischer(4; '15), this condition may be explained by stating that, since the blood has become chronically acid (see also Koehler, etc., 10), its proteins are able to absorb and hold extraordinarily large amounts of water.



Figs. 1 and 2 Figure 1 shows the general relations of the mesonephros, *M*, to other body structures. Figure 2 shows the oil-immersion appearance of the mesonephros.

RELATION BETWEEN HEMOGENESIS AND RESPIRATION

As stated above, the investigation of this point was the impelling motive for the present study. Figures 3 and 8 show a condition found in five of the embryos. Circulation had stopped, but both arteries and veins were packed with red blood cells. The amount of blood present suggests unusual hemopoietic activity. As indicated by the stippled areas of figure 3, this embryo had three distinct areas of blood congestion. Figure 8 is taken from a section through the same embryo in the plane indicated by the transverse line in figure 3. In this embryo the anterior and posterior cardinal veins were of enormous size and the posterior cardinals had fused ventrally. Figure 8 shows that the lower portion of the sinus

so formed, i.e., the portion next the yolk, was packed with blood, while the superficial portion was only partially filled. This indicates that the enormous size of the vessel could not have resulted from hemorrhage.

No endothelial lining could be distinguished on some portions of the wall and red blood cells could be seen in the adjacent mesenchyme. Probable cases of transformation of wall cells into erythroblasts were numerous and rendered even more likely by the fact that the blood contained a great many small, basic staining cells with large nuclei, which cells were interpreted as erythroblasts.

The remaining embryos contained large sinuses only partly filled with blood, as shown in figures 1, 5, and 6. Like the first group, their blood contained a large number of erythroblasts. Many of the fully differentiated erythrocytes were undergoing hemolysis.

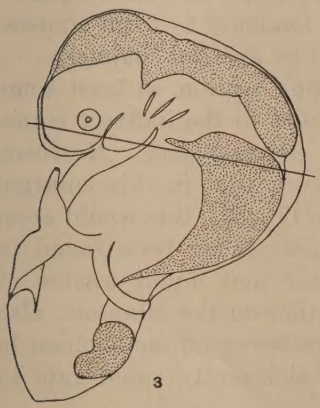
It is not within the purpose of this paper to decide the origin of all blood cells. For bibliography and discussion of theories on this subject, the reader should consult Cunningham, Sabin and Doan(2; '25), in which paper a very complete summary of the subject is given. However, as stated in the introduction, a previous study had suggested that cells in the walls of enlarging blood vessels might play a part in hemogenesis, and we will attempt to clear up this point.

The extraordinary amount of blood in some of these embryos may have been produced by unusual activity in the hemopoietic areas described by Danchakoff in the extra-embryonic area. It is interesting to note in this connection that two presumably normal eggs, incubated for ninety-six hours, were coated with water-glass and returned to the incubator, where they remained five days longer. At the end of that period, the only living portion of the embryos was a peripheral ring of the area vasculosa. Hemogenesis was still proceeding vigorously in that region.

It is also conceivable that increase in hemogenesis might be effected by mitosis of erythroblasts in the blood stream itself. This possibility cannot be denied, but it is perhaps

significant that not a single erythroblast has been found undergoing mitosis in the blood of these embryos.

Figure 4 shows an instance of the derivation of erythroblasts from the ventral wall of a postcardinal sinus similar to the sinus shown in figure 1. This example is extraordinarily clear. Dozens of other instances were found, but all were susceptible to the same objection—can one be certain that the erythroblasts are not merely lying very close to the wall and not derived from it at all? In this instance the objection just stated can hardly be raised. The section was



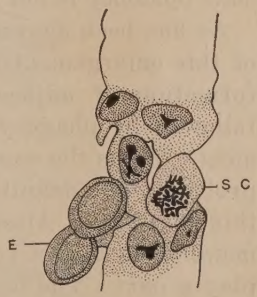


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Fig. 3 Ventral view of embryo, showing areas of blood congestion, stippled, and the plane of section of figure 8, by the transverse line.

Fig. 4 Hemogenesis in sinus wall. *SC*, stem cell; *E*, erythroblasts.

only 3 μ in thickness, the differentiation was good, and observation was made by means of a 1.9-mm. apochromatic oil-immersion lens on a Bausch & Lomb binocular fitted with 12.5 compensating oculars.

There would seem to be no question about the derivation of these cells, for the embryo was devoid of circulation at the time of fixation and no other blood cell was within 15 μ of the erythroblasts pictured in the same section. The very space from which these erythroblasts had migrated and a stem cell undergoing mitosis to replace them were both pres-

ent as illustrated. If this case is not adequate proof of the origin of erythroblasts from the walls of enlarging blood vessels, millions of instances would fail to convince.

RELATION BETWEEN DEVELOPMENT OF THE CIRCULATORY SYSTEM AND RESPIRATION

The general effects of suffocation on the circulatory system are at first rather surprising. A vigorous circulation is in no case produced, but a tendency to the formation of enormous sinuses within the body is displayed (figs. 5 and 6). The anterior and posterior cardinal veins and the omphalomesenteric veins show the greatest tendency to form sinuses. This tendency is but little displayed by arterial channels.

As has been shown in the preceding section, at least some of this enlargement may be attributed to the indirect transformation of adjacent cells into erythroblasts. Necrosis, followed by phagocytosis, may play a part in this enlargement. From the work of Maximow(17; '25), this would seem probable, but definite cases have not so far been found in this material. Absorption of water and liquid wastes of metabolism, as set forth in the section on the allantois, also play a part. The heart is unable to carry on metabolism in this toxic, anoxemic medium at a sufficiently rapid rate to permit it to function.

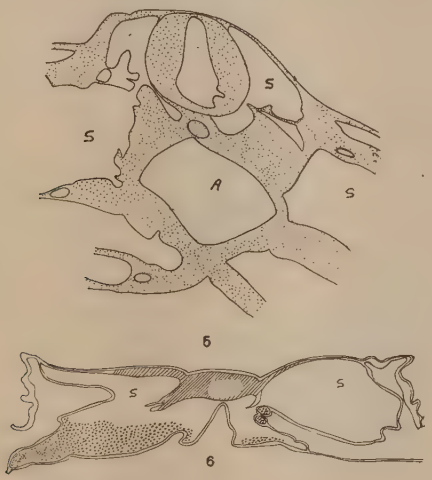
RELATION BETWEEN CELLULAR PROLIFERATION AND RESPIRATION

It has already been stated that all of these embryos showed a degree of cellular degeneration. This ranged from a relatively small amount in such an embryo as pictured in figure 3 to almost entire cytolysis as pictured in figure 14. Normally active cells were found contiguous to degenerating areas. This was very striking in certain areas of the neural tube in which degeneration had but little exceeded the region of the floor plate. In such regions the germinal cells of His at the very border of the degenerating area were undergoing apparently normal mitosis. Figures 9 to 12 show cases in which that growth had assumed abnormal proportions. A

single instance has so far appeared in which a wholly anomalous and very enormous epithelial proliferation had developed. This is figured in 13 and 14.

NECROSIS

The necrotic material appeared as a mass of cell fragments, free, rounded cells, apparently partially plasmolyzed, and droplets or pellicles of material which took a very deep basic stain. Necrosis apparently began by a rounding up and



Figs. 5 and 6 Enormous sinuses in cross-section. *A*, aorta; *S*, sinus

detachment or plasmolysis of the whole cell. Masses of such cells were characteristically found extruded from the wall of the neural tube into its central canal. Accompanying or preceding detachment of the cell, droplets appeared on the surface of the nucleus. This condition is illustrated in figure 7. From their appearance these droplets must have been extruded from the nucleus. This view is supported by the work of Ludford(14; '25), who showed that the nuclei of cells growing in vitro extrude particles, presumably nucleoli, by the rupture of the nuclear membrane. However, it cannot

be denied that the droplets on the nuclei in this material may have been formed in situ.

The initial detachment of the cells was followed by their fragmentation and apparently by the union of several of the droplets described above. Excepting the droplets, the fragmented cells stained with acid stains. These droplets were shown to be of a lipoid nature by the fact that they stained dark brown with 1 per cent osmic acid. They stained a very brilliant red with safranin, retaining the stain after all the safranin had apparently been extracted from the rest of the

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Fig. 7 Fat-droplets formed on surface of a necrotic nucleus

section. This is characteristic of the fat-droplets in the yolk as well. The droplets would not stain with sudan III after dehydration with alcohol. This stain has not yet been used with frozen sections. These droplets are probably homologous with the 'inclusions' of Maximow's material(15; '25), though he ascribes a protein nature to these inclusions because they stain with basic dyes.

The detritus must have been soluble in the blood plasma or have served as food for the remaining living cells, else there would have been a much larger amount of detritus in such embryos as those figured in 5 and 6.

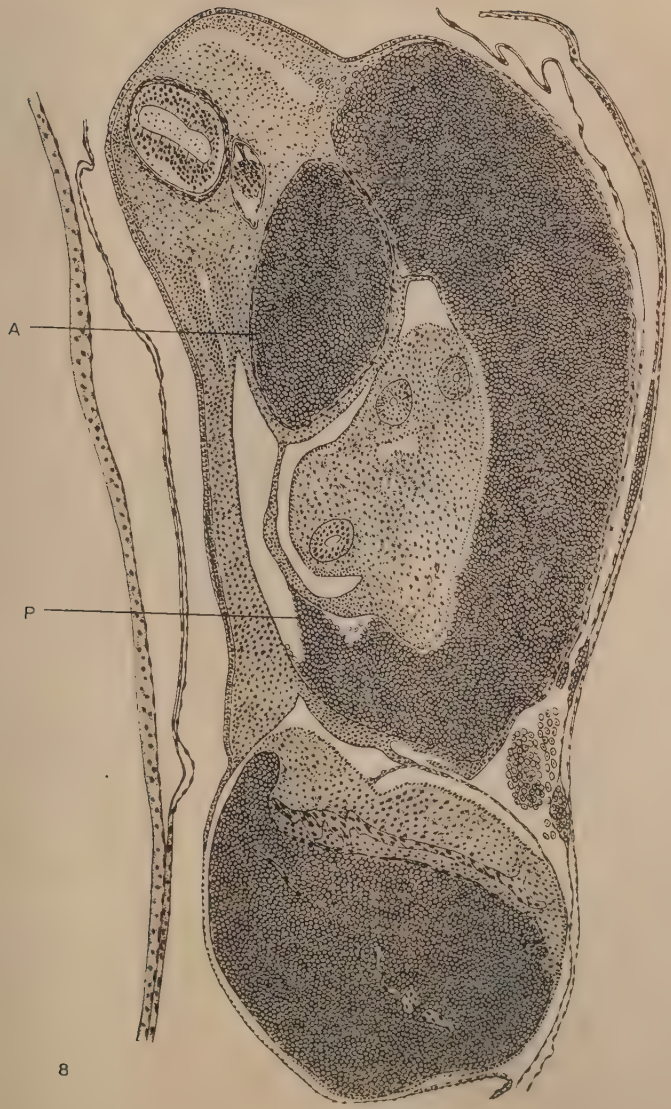

Fig. 8 Cross-section of embryo shown in figure 3. *A*, aorta; *P*, postcardinal sinus.

CELLULAR PROLIFERATION

The fact of cellular proliferation at the time of fixation of the material is considered as definitely proved by the presence of mitotic figures. Whatever tissue retained its optically normal state showed at least normal growth. Total growth had in no case reached its normal proportions. Body length averaged about two-thirds that of normal embryos. However, several of the embryos had normal-appearing limb buds, showing that differentiation was proceeding at approximately the normal rate in the living remnant.

Local, abnormal growths were present in three embryos to a sufficiently striking degree to warrant their more detailed description. These growths are shown in figures 9 to 14. Figure 10 shows a cross-section of an embryo with a marked dorsal enlargement of the neural tube accompanying necrosis of the lateral portions of the floor plate. Figure 9 shows the appearance of the shaded area 'A' under the oil immersion. It will be noted that mitotic figures are concentrated in a small area in figure 9, while the peripheral region of the enlargement consists of apparently normal resting cells. Figures 11 and 12 show a cross-section of another embryo in which the floor plate had entirely degenerated. Figure 12 is a drawing with oil-immersion lens showing a relatively large area of rapidly proliferating cells moving downward into the central canal from the dorsal portion of the alar plate. The appearance of both these sections strongly suggests a stimulative effect on the normal cells by the necrotic cells. Certainly, the necrotic cells must have served as food for the living cells.

Figures 13 and 14 show a cross-section of an embryo which had undergone almost total necrosis. Only the epithelial covering of the body and a small fragment of the roof plate of the neural tube were, apparently, alive. This embryo had developed an anomalous proliferation of the cells of the epithelium to form a mass a tenth the volume of the body of the embryo! The portion of this mass drawn in figure 13 shows it to be composed of many more or less rounded cells

embedded in a matrix, the exact nature of which is undetermined.

As stated in the section on methods and materials, the results obtained by the water-glass treatment have been corrob-

Figs. 9 and 10 Figure 9 is oil-immersion appearance of area *A* in figure 10. Figure 10, *G*, gut; *S*, sinus; *N*, notochord. Stippled area necrotic.

orated by the modifications produced in an embryo obtained by placing eggs in a bottle containing besides the eggs about 50 cc. of air per egg. The one embryo obtained by this method that has thus far been sectioned is essentially like the embryos

obtained by the water-glass treatment, and thus merits no general discussion. It bore four distinct dorsal epithelial proliferations, two of which are shown in figure 15, since their form is somewhat different from the growth figured in 13 and 14.

DISCUSSION

Cessation of heart beat is soon followed in the adult vertebrate by cessation of life phenomena in all the component

Figs. 11 and 12 Figure 11 is oil-immersion appearance of area *A* in figure 12. Figure 12, *Ao*, aorta; *N*, notochord; *S*, sinus. Stippled area necrotic.

structures of the body. But that the intervening period may be prolonged indefinitely is abundantly proved by the great mass of work on adult tissues and cells in vitro. The function of the circulating tissues, the blood and lymph, is to carry

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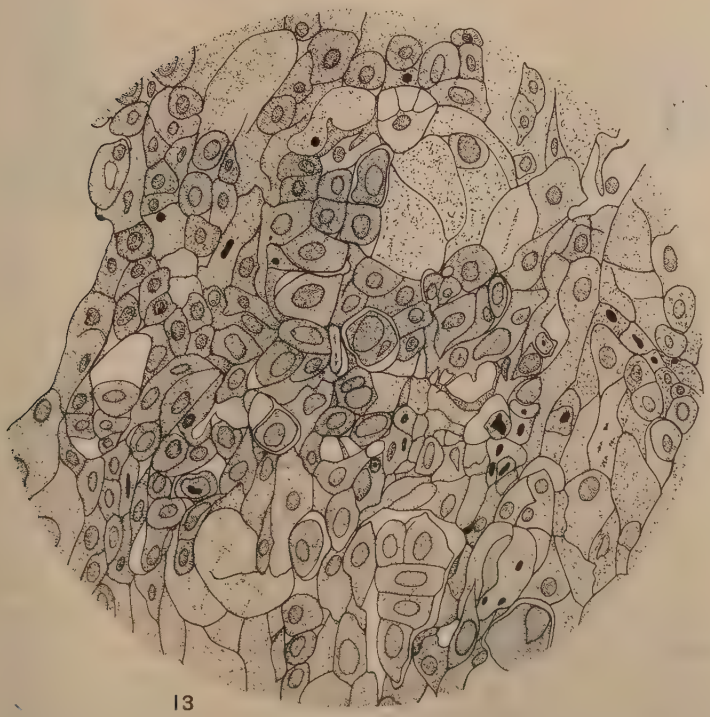

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Figs. 13 and 14 Figure 13 is oil-immersion appearance of a portion of *A* in figure 14. Figure 14 is from a cross-section of an almost wholly necrotic embryo with a large, anomalous, epithelial growth, *A*.

oxygen and food to the tissues, and waste material from them. Let us state it a bit differently; the circulating tissue is the agent by means of which the metabolic balance of the adult vertebrate body is maintained. Cessation of the circulation will lead to a struggle for existence between the component cells of the body. In the adult body cessation of circulation soon leads to the production of substances toxic to all the cells of the body, and their death follows. But will local disturbances of this balance in the adult produce the same result? Obviously not, for we know that injuries are followed by at least partial regeneration. Nor, as the material described shows, does death of the component cells necessarily follow

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Fig. 15 Shows two similar epithelial outgrowths from the dorsal surface of an embryo incubated in a small amount of unchanged air.

cessation of heart beat in the embryo. But partial disruption of the normal relationships between various cells and tisssues does occur in both the latter instances.

Let us first consider the embryos described in the preceding sections of the present paper. We have seen that the superficial cells of the embryo were able to use the little oxygen that penetrated to the embryo and thus remained alive after the death of the deeper tissues. This superficial growth, accompanied by deeper necrosis, is itself sufficient evidence that exclusion of air was not complete and that the effects noted were not due to toxicity of the water-glass. It has already been noted that the same effects are produced,

though not so quickly, by incubating the chick in a very limited amount of unchanged air. Were it not for the cases of abnormal cellular proliferation and of abnormal hemopoietic activity, little more would need to be said. But abnormal growth would seem to require abundant oxygen, and an explanation must be given for this seeming paradox.

Normal growth of the vertebrate, after differentiation of organs, is controlled by an interdependent system of hormones produced by the glands of internal secretion and distributed by the blood. Before circulation is established in the embryo and in any case in which circulation is blocked, such hormones could not circulate readily, and therefore any growth in such instances must be attributed largely to local factors. The establishment and maintenance of a complete circulation must be viewed as essential to normal regulation and correlation of growth.

When the circulation of any region is blocked, products of metabolism must remain in that region. The cells of the region must assume at least temporary anaerobic respiration in order to survive. In general, this will follow the anaerobic portion of the cycle given by Meyerhof(18; '24), for muscle. Surviving cells must become dependent on the death of neighboring cells for food. Any stimulus to growth must be locally produced. Growth will be anarchistic, unregulated by the body in general, until circulation is reestablished.

L. Loeb(13; '14) has isolated an ovarian hormone of a lipoid nature, responsible for the cyclic growth of the female generative organs. J. Loeb(11; '03) has produced parthenogenesis by treating Arbacia eggs with fatty acids and hypertonic salt solution. Tumors have been produced by the application of tar. Many abnormal growths have been produced by various sorts of chronic irritation or injury. Now injury causes the death of cells followed by autolysis with consequent formation of neutral or weakly acid lipoid substances and ammonia by deamination. It would appear that certain lipoid substances acting with a hypertonic medium must act as stimuli to local growth.

Warburg(20; '24) has proved that anoxemic chick-embryo tissue produces very large quantities of lactic acid. The work of Koehler, Benell, Behneman, and Loevenhart suggests that the intercellular medium is for a time hypertonic, as does the absence of intracellular swelling in our own embryos. Now the production of lactic acid in quantity is a certain index of a high percentage of anaerobic respiration. The embryos used in our studies and the fragments of embryos used by Maximow(17; '25) show exactly the local anarchistic growth one would expect under such conditions. This local uncorrelated growth proceeded until all the cells were killed by the acidity of their own products.

In case of injury, such growth must become active in order to effect repair. In case cells so produced should penetrate and block blood vessels growing into the tissue or in case of continued or repeated injury, circulation would not become complete, and a malignant growth or tumor would result. Warburg supposes that any circumstance, be it pressure, bacterial action, sclerosis of arteries, or whatever it may that produces oxygen deficiency, will cause cells to assume anaerobic respiration and will, if the condition becomes chronic, produce cancer. The present paper indicates that his prediction may be capable of proof, and it should be investigated with all thoroughness. Certainly, our own embryos are in accord with this supposition.

In general, we must conclude that the death of cells produces a substance or substances of a lipoid nature. As long as the circulation of the region is imperfect, this substance will accumulate and stimulate growth in neighboring cells of the same tissue. It seems probable from the recent work of Gye that cancers produce such a stimulating substance of a very specific nature. In view of the general behavior of tissues in vitro, does not their growth present a similar phenomenon?

CONCLUSIONS

1. Normal respiration is essential to normal circulation in the chick embryo.

2. Suffocation of the chick embryo brings about the formation of large sinuses.

3. Erythroblasts are developed from the wall cells of enlarging blood vessels.

4. Retention of metabolic products causes death of the deeper tissues of the embryo.

5. Certain of the more favorably placed cells are able to utilize the dead cells as food and continue to live.

6. Such cells must assume anaerobic respiration and any resulting growth must be anarchistic.

SUMMARY

This paper presents a new approach for the study of experimental embryology in the chick. Though the technique is still crude, it promises to give important and far-reaching results.

In closing, I wish to express my thanks for the great interest and careful criticism given this work by Dr. G. L. Houser and Dr. F. A. Stromsten, under whose direction this research has been carried on.

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CONGENITAL HYPERTROPHY OF THE EYE IN AN ALBINO RAT

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SEVEN FIGURES

Of the 50,000 rats which have been reared in the animal colony of The Wistar Institute, there have been very few cases of marked enlargement of the eyeball. Small-eyed rats have appeared at different times and in various strains, and are not unusual. Also, cataracts and other degenerative changes in the eye associated with defective dietaries were not infrequent in the earlier years of the colony. But the condition of increased size of the eye is apparently rare. Miss F. L. Duhring, curator of the animal colony, recalls but two examples, and the present case is the most marked that has been noticed.

This animal was first observed when it was quite young, soon after the disjunction of the eyelids. Attention was drawn to it, because of the unequal size of the two eyes. While the left eye was of normal size, the right eye was larger and protruded to a noticeable degree. The appearance at about three months of age, when the animal was examined, is shown in figure 1.

INSPECTION

The two eyes contrasted strongly in their coloration. The left normal eye was of the usual pale yellowish-pink color, while the large right eye was bright red, verging on vermilion. In diffuse daylight the left normal eye showed a pinpoint pupil, as is characteristic in albino rats. In the right

eye no iris or pupil could be discerned and there was no change in different intensities of light. Instillation of atropine solution in the two eyes gave the following results: The right eye gave no response to the mydriatic, while in the left normal eye the pupil dilated widely in seven minutes. This, according to our previous observations, falls within the normal time range for this reaction in the albino rat.

Ophthalmoscopic examination after instillation of atropine

In the left normal eye, when the pupil became fully dilated the media were found to be clear, and the details of the fundus, including optic disc and retinal vessels, could be seen to be normal. In the right eye there was a bright red coloration. The media were so cloudy in appearance as to prevent a view of the fundus.

Autopsy

Albino rat, male; body weight, 155 grams; brain weight, 1.729 grams; body length, 182 mm.; tail length, 144 mm.

The left normal eye was very nearly spherical, as is normal for the albino rat eye, with an anteroposterior diameter of 6.12 mm. and a transverse diameter of 6.37 mm. The right eye was elongated and distinctly enlarged. The greatest diameter was the anteroposterior, measuring 8 mm., while the transverse diameter was 7.25 mm.

The left normal eye weighed 0.131 gram in the fresh condition. This figure is to be compared with 0.12, the weight given by Donaldson ('24) for the eyeball of an 150-gram male albino rat. The right eye weighed 0.218 gram, and was therefore 66 per cent heavier than the left.

After being weighed, the eyes were cut open and the lenses removed. No differences were detected in the size or shape of the two lenses. They measured 4.5 mm. in the transverse, and 3.75 mm. in the anteroposterior diameter. This gives the ratio of 1 : 1.2, which is practically normal. F. Treacher Collins ('22) gives the transverse diameter of rat lens as 4.5 mm. and the anteroposterior as 4 mm. He does not specify

the variety of rat or the size. The eyeballs and lenses were preserved in Bouin's fluid.

The optic nerve coming from the large right eye was the smaller, and the chiasma was asymmetrical. The left anterior colliculus of the quadrigeminal plate was slightly smaller than the right.

Fig. 1 Photograph of albino rat, showing the right eye enlarged and the left eye of normal size. × 2/3.

Microscopic examination

Equatorial sections of the enlarged and normal eyeballs are seen in figures 2 and 3. In the normal eye the corneal segment forms nearly half of the perimeter of the eyeball (175° : 185°). In the enlarged eye the relative lengths of corneal and scleral segments are the reverse of this (185° : 175°). The most noticeable structural differences are seen in the iris, ciliary body, and cornea. In figure 2, immediately posterior to the cornea, is seen a thin membrane (*E*) which, on microscopic examination, is found to extend over the

entire posterior surface of the cornea. Anteriorly, a narrow space separates the membrane from the cornea, but laterally the two are in close contact. As the membrane is followed toward the ciliary region (fig. 6), it appears to split, one part of it running beneath the base of the anomalous iris and ciliary body to meet the choroid, with which it seems to be continuous. The other part of the membrane curves along the anterior surface of the iris tissue, and soon becomes indistinguishable from the adjacent tissue. This membrane is made up of delicate connective-tissue fibers and cells, with capillaries abundant in some parts of it. From its position and structure, it probably represents the persistent pupillary membrane, and the narrow space between the membrane and the cornea is the anterior chamber. Comparison of the cornea in figures 4 and 5 show that both the entire cornea and its anterior epithelium are thinner in the larger eye (*C*) than in the normal. This thinning of the cornea, taken in connection with the enlarged anterior segment of the eye, shows that there has been increased pressure within the eye. At *F*, figure 6, is seen the exudate which occupies the enlarged posterior chamber. This shows fine fibrin threads, a granular precipitate, scattered groups of red blood cells, and an occasional leucocyte.

The comparative conditions of the iris and ciliary body are shown in figures 6 and 7. Both of these structures are notably defective in the enlarged eye and consist principally of highly vascularized connective tissue. The region *G*, figure 6, representing the iris, consists only of vascular connective tissue and lacks entirely the pars iridica retinae on its posterior surface. Likewise, the ciliary body is made up chiefly of blood vessels and connective tissue, with practically no ciliary muscle. The pars plana (*P*) is greatly elongated as contrasted with this part (*P'*) in the normal eye, and along it is found the pars ciliaris retinae as a continuous double cell layer. There is the appearance, in some sections, of slight projections simulating ciliary processes, but these are very slight, and hardly comparable with the normal processes

seen at *H'* in figure 7. The epithelial cells of the pars ciliaris retinae continue over the abortive ciliary processes, but are not so regularly arranged as in the normal eye. In addition, one notes the presence of large thin-walled blood vessels (*K*), which have their origin, as seen in some sections, from the ciliary region. At *L*, figure 6, however, the retina begins in the usual manner and has, in general, a normal appearance.

DISCUSSION OF OBSERVATIONS

The condition is evidently a congenital one, originating at the time of differentiation of the iris and the ciliary body, probably between the eighteenth and twenty-first day of fetal life. Examination of the developing eye of the eighteen-day white rat fetus shows the pars caeca at the margin of the optic cup, but as yet no indication of the pars ciliaris and pars iridica retinae. There is a very distinct capsula vasculosa lentis, continuous about the entire lens, and the distal portion of the capsula (membrana pupillaris) is in close apposition with the mesenchymal tissue of the developing cornea and with the inner side of the pars caeca retinae.

In the twenty-one-day fetus the anterior chamber is clearly formed, and there is an indication of the development of the pars caeca into the pars iridica and pars ciliaris retinae. The tunica vasculosa lentis is still apparent, encircling the entire lens, and in the pupillary region it is an attenuated membrane containing thin-walled blood vessels at intervals. The membrane also receives a thin fibrous continuation from the mesenchymal tissue on the outer side of the pars iridica retinae.

From a comparison of the condition seen in the hypertrophied eye with this stage of development, it is apparent that the time at which the divergence from the normal processes began must be placed at this period. The mesenchymal tissues are the ones most affected. The pupillary membrane has persisted, while the iris and the ciliary body are incompletely developed. The tissues at the angle of the anterior chamber are also involved. Thus neither the forma-

tion nor the drainage of the aqueous humor could proceed in the normal manner. Evidently the rate of drainage was less than the rate of formation and the fluid accumulated in abnormal amount. This increased amount of fluid enlarged the posterior chamber and pressed the persistent pupillary membrane forward against the cornea. While no significant histological differences were detected in the retinae of the two eyes, the fact that the optic nerve from the enlarged eye was smaller than that from the normal eye shows that there must have been some alteration. As there is practically complete decussation of the optic nerve fibers in the rat, it is not surprising that the left anterior colliculus, receiving the smaller right optic nerve, should likewise be smaller than the right anterior colliculus.

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PLATE 1

EXPLANATION OF FIGURES

2 Transverse section of enlarged right eye ball, passing through the optic-nerve entrance. This shows the relative increase in size of the cornea, as compared with the scleral segment. × 10.

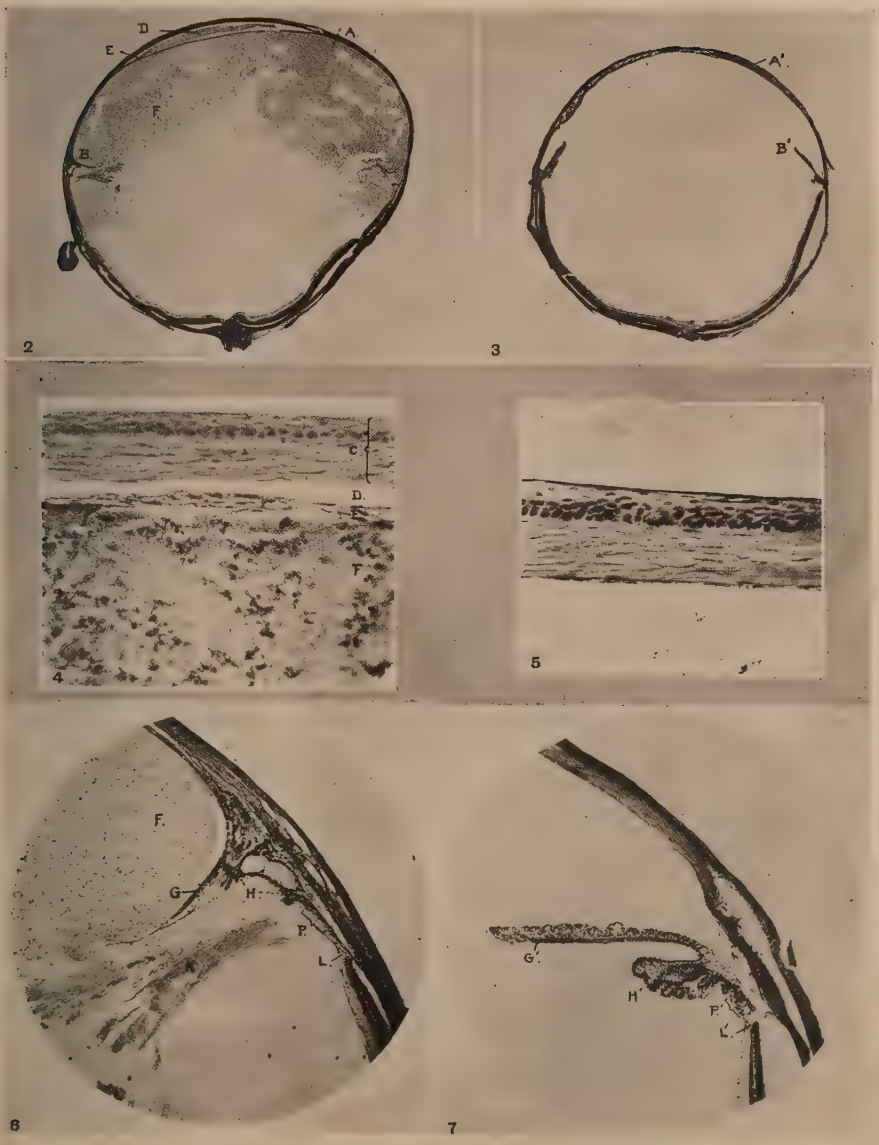
3 Transverse section of normal left eye ball, passing through the optic-nerve entrance. × 10.

4 Microphotograph of region *A* in figure 2, showing thinned cornea (*C*) and vascular membrane (*E*), with narrow anterior chamber between (*D*). Posterior to the vascular membrane is a fibrinous exudate (*E*), containing scattered groups of erythrocytes. × 210.

5 Microphotograph of region *A'* in figure 3, showing cornea of normal eye. × 210.

6 Enlarged view of region *B* in figure 2, showing the maldeveloped iris (*G*) and ciliary body (*H*). *F* indicates the exudate occupying the distended posterior chamber; *K*, ciliary vascular plexus; *L*, beginning of retina; *P*, pars plana elongated. × 52.

7 Enlarged view of region *B* in figure 3, showing normal iris (*G'*) and ciliary body (*H'*). *L'*, beginning of retina; *P'*, pars plana. There has been some separation of the parts in the preparation of the section. × 52.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NOTES ON A CORRELATED COURSE IN ANATOMICAL SUBJECTS

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After several years of discussion and planning, the Department of Anatomy of the University of Pittsburgh School of Medicine abandoned the teaching of separate courses in gross anatomy, microscopic anatomy, embryology, gross neuro-anatomy[1] and organs of special sense at the beginning of the university year 1924–1925. In place of these, a single closely correlated course in these subjects was given. The results of this educational experiment were, in the main, so successful that this type of course has been adopted as our teaching program.

There is no thought of claiming any originality for the idea.[2] Nevertheless, the number of inquiries for specific information relative to the course in actual operation here indicates such a definite interest in this method of anatomical teaching that a brief summary of it seems justified.

During the preliminary discussion, it became evident that what could be given here would have to be a compromise between our conception of an ideal correlated course and the somewhat unusual subdivision of the time allotted to us on the schedule. However, as our conception of an ideal correlated course would probably be quite different from that of others, no discussion of this subject seems appropriate here.

The course as given in 1924–1925 manifestly required alteration, chiefly by transposition of subject-matter. The changes

[1] The study of the details of the finer anatomy of the nervous system is here given in the second year.

[2] We are greatly indebted to Dr. E. R. Clark for his discussion of his experience with a correlation course at the University of Georgia.

made have cleared up the principal difficulties encountered and have furnished the general operating plan used this year and described below.

The course as a whole is divided into six parts: 1) Introductory histology and embryology, 34 periods (three hours each). 2) Head and neck, including superficial chest, back, gross neuro-anatomy, and organs of special sense, 85 periods. 3) Thorax, 14 periods. 4) Abdomen, 26 periods. 5) Pelvis and perineum, 23 periods. 6) Extremities, 40 periods.

The section which comprises the work on introductory histology and embryology is carried out entirely in the microscopy laboratory. The work covers the study of the cell and its activities; the germ cells, studied as specialized cells; maturation, fertilization, cleavage, and formation of the germ layers, and the histology and histogenesis of the elementary tissues. This constitutes an introduction to the study of organs as outlined below.

Dissection is begun with the section on the head and neck. The microscopic structure and the development of the various organs, etc., are studied as they are encountered. As the students work two to a cadaver in the dissecting room and each pair is assigned to adjacent desks in the microscopy laboratory, the frequent transition from work in one laboratory to that in the other is readily accomplished. Students are encouraged to study in the microscopy laboratory pieces of tissues and organs taken from their cadavers.

When the dissection of the head and back has reached the logical point, the study of the gross structure of the brain and cord is undertaken. About half the cadavers used in the laboratory retain the brain in situ. Additional, formalin-preserved brains are issued for more thorough study, as those left in the cadavers are used primarily for their relations to the skull, meninges, etc.

The study of the thorax, abdomen, pelvis and perineum is carried on in the same manner. The outline of the work on the thorax actually carried out, period by period, last year is appended as being a characteristic example of our

method. The dissection of the extremities is utilized largely as a practical test of the student's ability to dissect, observe, and correlate.

The purpose of the course is to give the student a more comprehensive and closely knit knowledge of the structure and development of the human body than was possible with the older method. Daily observation of each student's work in the laboratory, the quizzes and examinations given, clearly demonstrate that this was accomplished. The students possessed a much better comprehension of the structure and

TABLE

Outline of work covered in the study of the thorax in 1924–1925

Period	1.	Dissection of intercostal muscles and spaces.
Period	2.	Study of distribution and relations of pleurae and peritoneum to chest wall. Removal of ribs and sternum.
Period	3.	Study and dissection of lungs, one in situ, one removed.
Period	4.	Histology of larynx, trachea, and primary bronchi.
Period	5.	Histology of lungs and development of respiratory system.
Period	6.	Dissection of superior mediastinum.
Period	7.	Dissection of pericardium and heart in situ, superficial.
Period	8.	Dissection of heart and study of cavities, valves, etc.
Period	9.	Histology and embryology of heart.
Period	10.	Dissection of posterior mediastinum.
Period	11.	Histology and embryology of thymus and thoracic duct.
Period	12.	Practical examinations. Review and study.
Period	13.	Practical examinations. Review and study.
Period	14.	Written examination.

function of the human body at the end of the admittedly imperfect course given in 1924–1925 than has been the case in any year heretofore. However, few of the students had the same knowledge of details of structure as has been exhibited under the older system. The reader is left to judge which is the more important: a genuine working knowledge of the body, or a rather facile (and, we believe, temporary) grasp of relatively unrelated details of structure.

A quite unexpected result of our experience with this type of course is that no time is saved by the correlation, for reasons not entirely apparent.

This type of course has, we believe, another important aspect touching the teaching staff. It is our custom to divide up the parts among the staff, one member being chief and others assistants, in such a manner that each member of the full-time staff serves as chief in one or more parts and as assistant in others. In the building up of an anatomical staff, the greatest problem concerns the training of younger men so that they have a comprehensive teaching experience in all phases of the subject. A course of the type here outlined provides probably the best method for training younger instructors that could be devised. Perhaps it should be noted here that such a course also makes it possible to free members of the staff seriatim from all teaching obligations, thus providing periods of continuous time for thoughtful study so essential to research.

The success of this experiment in correlating the anatomical subjects leads us to believe that the time will come when, in spite of difficulties which now appear enormous, an actual close coordination of anatomy and physiology will be accomplished.

In conclusion, I wish to express my indebtedness to my colleagues, John C. Donaldson and J. S. Nicholas, to whose thoughtful consideration and active cooperation the planning and successful execution of this idea are largely due.

SUMMARY

1. A correlated single course in all anatomical subjects is not only possible, but, as judged by this experience, desirable.

2. By it the student gains a more coordinated working knowledge of anatomy, though less detailed information, than by the older type of separate courses.

3. Such a course does not save time by the elimination of redundancies.

4. Such a course is nearly ideal for training younger instructors in anatomical teaching.

IRON HEMATOXYLIN AS A MYELIN SHEATH STAIN AND NEUTRAL RED RIPENED BY COLON BACILLUS AS A NERVE-CELL STAIN

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ONE PLATE (TWO FIGURES)

The following staining methods grew out of the difficulties that I experienced in studying lesions of the corpus striatum and secondary degenerations in a number of human brains furnished to this university by the Binghampton State Hospital. It became necessary to find some method by means of which this old formalin-fixed material could be easily cut and examined, both as to the integrity of the myelin sheaths of certain tracts and the integrity of the cells in the region of the lesions. The older methods of mordanting in potassium dichromate, Muller's fluid, fluorochrome, etc., were found to be tedious and usually resulted in uneven staining, difficulties in differentiation, a loss of selective staining of the cells and a brittleness of the specimen which caused difficulties in cutting and handling. The large size of the sections in this region increased these difficulties.

The following iron-hematoxylin method was found to avoid these undesirable features of the older Weigert methods. Sections stained by this method were demonstrated at the meeting of the American Association of Anatomists in March, 1925 (fig. 1).

THE IRON-HEMATOXYLIN METHOD OF STAINING

The iron-hematoxylin method of staining tissues was described by Heidenhain ('92). It has since been extensively used as a histological and cytological stain with but few

modifications. A good general discussion of this staining method is found in Lee's "The Microscopists Vademecum," Guyer's "Animal Micrology," and in other books on microscopic technique. Although iron-hematoxylin is sometimes mentioned as a neurological stain (Houser, Lee, Nageotte, Spielmeyer, and others), it does not appear to be used to any extent in this country.

In this laboratory iron-hematoxylin was first used to stain seven human pathological brains, parts of which, from previous trials, could not be satisfactorily stained by the Weigert method or any of its modifications. The specimens had been fixed and preserved in formalin for a number of years. Without any mordanting, they were first cut into large blocks, embedded in celloidin, and sectioned 50 μ in thickness. They cut perfectly and could be handled roughly in a sieve in the staining process. Paraffin sections of the whole human brain furnished us by Spegall and prepared according to the method devised by him ('25) stained well with iron-hematoxylin. Excellent results have also been obtained with animal brains fixed in formalin and formol-alcohol whether embedded in paraffin or celloidin.

Unsatisfactory results followed the procedure ordinarily recommended for staining with iron-hematoxylin, namely, mordanting the sections four hours or longer in a 4 per cent solution of iron alum, staining overnight in a ½ per cent hematoxylin solution, and destaining in a 2 per cent iron-alum solution. This failure I attributed to overaction of the iron alum, which, when used excessively, tends to turn the cytoplasm and ground substance brown and opaque so that a clear differentiation cannot be made. Some modifications were found necessary to make it a satisfactory stain for myelin sheaths of nerve fibers.

The foregoing difficulties were avoided by, 1) shortening the time of mordanting to two hours or less in the 4 per cent iron-alum solution; 2) staining a shorter time (three to eight hours) in the ½ per cent solution of hematoxylin; 3) destaining only partially with the 2 per cent iron alum, and, 4) com-

pleting the destaining with a ½ per cent solution of hydrochloric acid.

In staining nervous tissue it was found that the iron alum destained the myelin sheaths quite slowly, while the background destained more rapidly and turned brown and opaque before the myelin was sufficiently differentiated. On the other hand, a weak acid solution was found to destain the myelin rapidly, while the nerve cells and neuropil differentiated more slowly. The successive use of iron alum and hydrochloric acid solution gave uniform and excellent results in differentiating sections stained with iron-hematoxylin.

The best results in destaining can be obtained in the following way: 1) Sections should be destained in a 2 per cent iron-alum solution for a few minutes until the larger fiber tracts are outlined quite clearly, but not until all the blue of the hematoxylin is completely extracted from the background substance. 2) The sections should then be rinsed in tap-water and flooded for a few seconds with ½ per cent solution of hydrochloric acid until the background becomes completely decolorized and the fibers stand out in great detail. 3) The sections should then be washed in running water for at least one hour to wash out thoroughly the acid and iron alum.

In sections properly destained by this method the background will be clear and white and the fibers will be stained a dark blue (fig. 1). The intensity of stain can be best varied by staining a shorter or longer time in hematoxylin, but not by varying the time in the mordant. A more finely differentiated section for microphotography or microprojection may be obtained by placing the sections, after staining, first into ½ per cent solution of hydrochloric acid for ten to fifteen seconds and washing, before differentiating in the iron alum and hydrochloric acid, as above described.

The advantages of this method of staining are the following. Specimens of any size can be embedded and sectioned before mordanting, and hence large sections are more easily cut and handled. The entire staining process requires but a few hours. The sections do not become brittle. The method

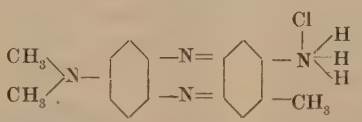
is applicable to old as well as fresh material following nearly any fixation. The results obtained are uniform, excellent in detail, and reliable. Sections stained with iron hematoxylin can be counterstained with neutral red or any other satisfactory stain for nerve cells.

The procedure for staining with iron-hematoxylin may be summed up as follows:

1. Fix parts of central nervous system (Flemming, Carnoy's, Bouin's, alcohol, formalin, formol-alcohol, etc.); dehydrate, embed in either paraffin or celloidin, and section.
2. Mordant sections two hours or less in 4 per cent iron alum. Formula: ammonio-ferric sulphate $(NH_4)_2\ Fe_2(SO_4)_4$.
3. Stain three to eight hours in $\frac{1}{2}$ per cent well-'ripened' hematoxylin.
4. Ordinarily destain in 2 per cent iron alum until larger fiber tracts become well outlined. (If very fine differentiation is desired, place sections in $\frac{1}{2}$ per cent hydrochloric acid for ten to fifteen seconds and rinse in water before destaining in iron alum.)
5. Rinse in water.
6. Place in $\frac{1}{2}$ per cent hydrochloric acid for a few seconds until background becomes colorless and fibers are well differentiated.
7. Counterstain with neutral red (if desired).
8. Dehydrate clear in xylene and mount in balsam.

NEUTRAL RED

Neutral red, the chloride of toluylene red, is a weakly basic dye. Conn gives to it the formula;



Johnston has pointed out the excellence of neutral red as a cell stain for the central nervous system. He states, however, that "the only means by which he has been able to prepare a satisfactory staining solution is by allowing the solution to

ripen for months or, better still, for years. Boiling and exposure to air hasten this ripening process."

Over a period of two years I have tried to prepare a well-'ripened' solution of neutral red. Several experiments, such as incubating at 70°C., boiling with glucose, addition of weak acids, alkalies, and various chemical reagents, failed to give the desired results. Solutions exposed to the air for several months with frequent boiling gave only partially satisfactory results.

I have, however, been able to prepare a solution of neutral red with excellent staining qualities in twenty to seventy-two hours by inoculating a 1 per cent solution with bacterium coli communis and incubating at 37°C. The stain prepared in this manner is highly selective for cell nuclei, nuclear membrane, nucleolus, Nissl granules, axone hillock, and the cell processes for a short distance beyond the cell. These structures stain a clear brilliant light red. The granules and nucleolus stand out most distinctly (fig. 2).

Michaelis quotes Rothberger and Wolff as saying that "Neutral red in aqueous solution is reduced by bacterium coli to a brilliant yellowish fluorescent substance." Because of this ability of B. coli to reduce neutral red, the dye is frequently used in combination with various culture media, as a test for B. coli. Regardless of the strength of the inoculation or the length of time incubated, I have never been able to reduce neutral red in aqueous solution to a yellowish fluorescent substance. Perhaps the reduction is too slight in this case to be perceptible without using special methods. There is no doubt, however, that neutral red is reduced by B. coli in culture media. Chamot and Sherwood have shown that this change brought about in neutral red by B. coli is a true reduction reaction which can be produced chemically by the use of sodium hydrosulphite ($Na_2S_2O_4$). However, I found that a partial reduction of neutral red in solution by the addition of very small portions of sodium hydrosulphite detracted from, rather than increased, the staining qualities of the solution. It seems quite evident, then, that the effect of B.

coli on neutral red which increases its staining properties is not due essentially to a reduction or partial reduction of the dye.

Since the action of B. coli on neutral red is not a reduction reaction, the following experiments were performed to determine, if possible, the real nature of the reaction. 1) A fresh 1 per cent solution of neutral red was inoculated with B. coli from a nutrient agar test-tube culture twenty-four hours old, and was found on plating to contain 900,000 B. coli organisms to each cubic centimeter of neutral red solution. Plates made from this solution after twelve hours' incubation showed that no active organisms were present in the solution. 2) Organisms killed by heating and then added to a fresh neutral red solution were found to be nearly as effective as the live bacteria. 3) A piece of potato improved the staining properties to a marked degree, but after a few days the dye was precipitated as a brownish-colored substance. The water pressed out of a potato, however, failed to 'ripen' neutral red, but precipitated the dye. This may indicate the presence of enzymes within the potato cell which ripen the stain, while other substances present are responsible for precipitation of the dye. 4) B. coli possess the property of curdling milk—a property which is also possessed by the enzyme rennin. There are on the market commercial products such as junket tablets used for this purpose. By adding one junket tablet to 75 cc. of 1 per cent solution of neutral red and warming gently for a few minutes, a quite satisfactory staining solution was obtained. The results were less favorable when a glycerin solution of rennin (Armour's) was used. This may be because the junket tablets contain substances from the gastric juice which aid in the reaction but which are not extracted by glycerin. 5) The enzymes pepsin and trypsin failed to ripen a solution of neutral red. These facts might all be taken to indicate *a*) that the transformation of neutral red into a satisfactory stain for nerve cells in these cases is accomplished essentially by an enzymic reaction. *b*) The enzyme is most effective as it is liberated by active, live B. coli, but is

also present in the body of the dead organism. *c*) The action of this enzyme is apparently quite similar to that of the enzyme rennin.

The most satisfactory means of 'ripening' neutral red is by the use of B. coli. The method is uniform and certain. The organisms seem to carry the process to just the right stage, no matter how strong the inoculation. Of the six solutions prepared in this laboratory, only one 1000 cc. stock solution, which was inoculated with a single loop of organisms from a two-weeks-old culture, required a second inoculation. The use of one twenty-four-hour test-tube culture of B. coli on nutrient agar slant to 50 cc. of 1 per cent neutral red solution gave no better results than did the inoculation of one-fourth of a test-tube culture to 1000 cc. of the neutral-red solution. The use of one fresh (preferably twenty-four-hour) test-tube culture for each liter of stain would be certain to give satisfactory results. Two 1 per cent stock solutions which failed to 'ripen' after repeated boiling and exposure to the air for seven months were 'ripened' successfully by B. coli in seventy-two hours. Experiments indicate that neutral red is thoroughly 'ripened' after twelve to twenty hours' incubation with B. coli, but I have made it a practice to incubate three days in order to be sure that the process was complete.

B. coli are easily isolated and should be obtainable without difficulty from any bacteriological laboratory or otherwise. In case B. coli cannot be obtained, the enzyme rennin is the best substitute. It may be had as rennet (rennin extract) or as junket tablets, which serve the same purpose. One junket tablet has been found to 'ripen' 75 cc. of 1 per cent solution of neutral red, which is about thirteen times the quantity required to curdle an equal volume of milk. If not enough of the enzyme is used, the stain is not completely 'ripened,' while too much causes a precipitation of the dye as a brownish-red substance.

Excellent Nissl preparations have been obtained with neutral red with 50 μ celloidin sections through the human

brain stem without removing the celloidin (fig. 2). Johnston has used much thicker sections. Paraffin sections through formalin-fixed animal brains gave equally good Nissl preparations.

The foregoing experiments have been made with Grübler's neutral red. Neutral red will follow fixation by Flemming's fluid, Bouin's fluid, acetic alcohol, osmic acid, formalin, alcohol, or formol-alcohol. Neutral red will not give satisfactory results following the use of potassium bichromate, either as a fixer or as a mordant. I have used formalin and formol-alcohol almost entirely and have had very satisfactory results with both fixation and staining.

The stain gives the best and quickest results if a warm $\frac{1}{4}$ to $\frac{1}{2}$ per cent solution is used. Thirty to sixty seconds is sufficient time for staining with a well-'ripened' $\frac{1}{2}$ per cent solution, or until the sections are dark red in color (the color of the staining solution). The best intensity of stain is that which requires two to three minutes for destaining in 75 per cent alcohol. Sections are destained in 75 per cent alcohol until but the faintest trace of pink is seen when the section is held toward the light. Sections must be carried as quickly as possible through the higher alcohols, to prevent over destaining, and into castor xylene or xylene as a clearing agent. Castor xylene is a satisfactory clearing agent, after which it is well to pass the sections through xylene before mounting. This procedure is essentially the same as that described by Johnston. If celloidin sections are used, a number of sections may be carried through at one time by the use of a perforated container, such as a small screen strainer or a tea-ball.

Johnston states that "Neutral red works admirably as a secondary stain after Cajal or Bielschowsky process on formalin or alcohol material." It will not follow Weigert, Marchi, or any other method in which potassium bichromate has been used.

Neutral red works beautifully as a secondary stain following iron-hematoxylin. Sections stained with iron-hema-

toxylin and counterstained with neutral red were demonstrated at the meeting of the American Association of Anatomists in March, 1925, although the neutral red used at that time had been ripened by aging and was only partially satisfactory. The faint pink of the background substance makes the fibers stand out even more clearly than before. The cells in some respects stain even better than when neutral red is used alone, for the cell nuclei, cell well, etc., retain the hematoxylin stain, while neutral red brings out the Nissl granules so that the result is a double stain for the nerve cell. The process of counterstaining with neutral red is the same as that described above for staining with neutral red alone.

Iron-hematoxylin and neutral red furnish a double stain which is excellent in preparing serial sections for laboratory study in neurology and for pathological diagnosis, because a study of both fiber tracts and cells can be satisfactorily made on a single series of sections.

These problems in staining have arisen incidental to the study of the connections of the corpus striatum; a problem which was suggested to me by Dr. J. W. Papez, to whom I am indebted for helpful suggestions. Dr. S. A. Goldberg furnished the cultures of B. coli; Mr. P. S. Prickett tested the fate of B. coli in inoculated and incubated neutral red solutions; Dr. J. M. Sherman suggested the use of potato and dead bacteria; Dr. E. M. Chamot suggested the use of sodium hydrosulphite, junket tablets, and rennin in conduct of the experiments. To these gentlemen I acknowledge my grateful indebtedness.

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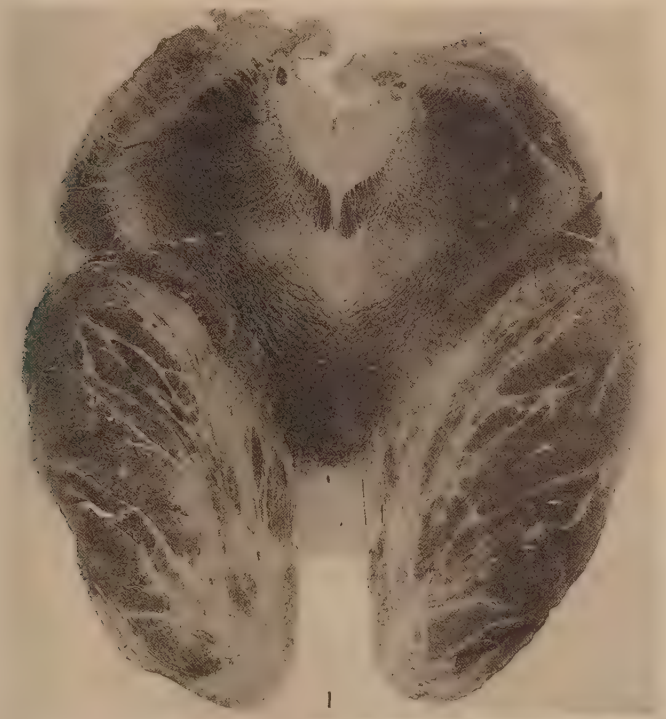
PLATE 1

EXPLANATION OF FIGURES

1 Microphotograph of a 50 μ celloidin section (celloidin not removed) through a pathological human brain which had been fixed and preserved for several years in formalin, mordanted and stained in section with iron-hematoxylin.

2 Microphotograph of a 50 μ celloidin section (celloidin not removed) through the hypoglossal nucleus of the same brain as figure 1, stained with neutral red ripened by B. coli.



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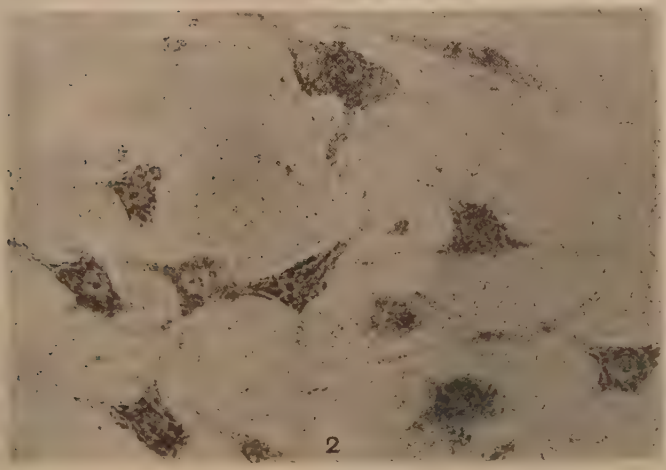

2

NOTES ON THE SALIVARY GLANDS

DOUGLAS REID
New Museums, Cambridge, England

ONE FIGURE

Are there ever tensors of the parotid fascia? The muscle indicated in the figure, present on the right side in an adult male, answers to the description of such a muscle, and I have observed an exactly similar muscle present on the left side in one other adult and bilaterally in a third.

It might be described as a transversus nuchae muscle prolonged on to the face.

It arose from the tendinous fibers of attachment of sternomastoid and trapezius muscles a short distance from the external occipital protuberance, and passed, as a fleshy slip, forward and somewhat downward below the pinna across the sternomastoid. At the anterior border of this muscle it ended in a tendon which was inserted into the parotid fascia. [I have also noted a muscle which arose from the superior curved line of the occipital bone adjoining the posterior fibers of insertion of sternomastoid. It passed downward and forward over this muscle and under cover of the great auricular nerve and external jugular vein to be inserted into the cervical fascia in front of sternomastoid, a little below the submaxillary gland.]

That the muscle may really be specially derived from the retrahens auriculam (auricularis posterior) was indicated by its receiving a nerve from the branch that supplied this muscle.

In this connection it is noteworthy that the transversus nuchae (Schultze) may be joined to the retrahens auriculam (Hallett).

I have also seen the retrahens muscle represented by two quite independent muscular slips. The upper and thicker of these had the usual attachments. The lower was a long band which arose from the outer part of the superior curved line of the occipital bone, extending its origin along this to a point $1\frac{1}{2}$ inches from the external occipital protuberance, and therefore into the region of the transversus nuchae. It passed forward over the fibers of insertion of sternomastoid to be inserted into the concha below the upper slip.

SPECIAL RELATIONS OF THE PAROTID GLAND

In an adult male a number of the lobules on the deep aspect of the 'facial process' of the parotid penetrated among the superficial fasciculi of the masseter muscle to which the gland firmly adhered. I have not seen this before and it must be rare. The parotid here was unusually large, although on microscopic examination it appeared healthy. The condition might be worth keeping in mind in cases of pathological enlargement of the gland requiring excision, as otherwise diseased parts might possibly be left behind.

Behind the masseter the part of the gland which lies directly on the mandible and the external (temporomandibular) ligament of the joint sometimes dips inward over the mandibular notch to come into immediate relation with the insertion end of the external pterygoid muscle.

THE SUBMAXILLARY AND SUBLINGUAL GLANDS

Special attention has been drawn to the fact that the submaxillary gland often extends downward beyond the limits of the digastric triangle. "Il n'est pas rare de la voir déborder d'un centimètre la grande corne de l'os hyoïde; Ricard a depuis longtemps attiré l'attention sur cette disposition, dont il ne faudrait cependant pas exagérer la fréquence (Ricard Bulletin Soc. Anat. 1889)"—Poirier's Anatomy, 1905. Symington again called attention to this. (Journal of Anatomy, 1912, vol. 46, p. 173). In an adult male the gland (superficial part) extended upward not only to lie over the styloglossus

muscle, but to lie external to the superior constrictor of the pharynx at its origin from the mandible. It covered the lingual nerve at this point.

On the other hand, bilateral absence of the submaxillary gland has been recorded as well as a gland "about equal in size to the normal sublingual," lying entirely above the mylohyoid muscle (Turner, Journal of Anatomy, 1870, vol. 4, p. 147).

In an old female I found what at first seemed to be two separate submaxillary glands *on each side of the neck.* Between and separating them lay the mylohyoid nerve and the submental branch of the facial artery (fig. 1), and the condition seemed to be correlated with the unusual position of the vessel. The higher of the two glands extended forward to the insertion of the anterior belly of the digastric muscle, and backward nearly to the point where the facial artery crossed the lower border of the mandible.

More careful examination showed that opposite the second premolar tooth on each side the mylohyoid muscle was absent at its origin.

It thus arose in two parts, anterior and posterior, separated by a gap of 1 cm. These parts met before reaching the median raphé, and thus bounded with the jaw a triangular orifice 1.5 cm. long with summit pointing toward the middle line.

Through this the gland protruded.

On reflecting the muscle, it was seen clearly that the gland was the sublingual. More than the lower half of this, and much the bulkier portion, lay below the 'diaphragma oris' and in contact with the inner surface of the mandible below the origin of the mylohyoid muscle. Here the bone presented a distinct fossa which took the place of a submaxillary fossa but extended farther forward.

The gland was not abnormally large and measured just over 1½ inches in length.

On microscopic examination, it appeared healthy.

Its upper part lay in a slight fossa on the jaw above the fore part of the mylohyoid muscle. For the most part it had the usual relations of the gland. The sublingual branch of the lingual nerve reached the gland on its deep and not on its superficial aspect. The deep part of the submaxillary gland was normal.

It was especially remarkable that the condition was not only bilateral, but almost perfectly symmetrical.

Moreover, the submaxillary gland, "which appears in embryos of the sixth week (13.2 mm.)" the sublingual appearing "at about the eighth week" (Keibel and Mall's Embryology, vol. 2, p. 348), had obviously been displaced downward or 'crowded out' of place by the sublingual. It did not come into contact at all with the inner surface of the mandible. Only at the angle of the lower jaw did it ascend slightly under cover of the bone, to lie in contact with the internal pterygoid muscle.[1]

Apparently the position of the submental artery was secondary. In Cunningham's Anatomy this artery is described as lying "on the lateral surface of the mylohyoid muscle, and medial to the upper part of the submaxillary gland." Gray also describes it in this position.

In relation to the gland, I find this artery usually follows the lower border of the mandible lying along, usually just above, the border between the osseous (superolateral) and superficial surfaces of the gland. In other cases it lies on the

[1] Only in one other adult (male) have I noted (on the left side) a submaxillary gland which presented no relation to the medial surface of the body of the mandible. Thus there was no submaxillary fossa. Le Double could only collect two recorded cases of its absence. The gland was an elongated structure about ¾ inch wide, and extended downward from the angle of the mandible along the anterior border of the sternomastoid. Its upper extremity only just got under cover of the insertion of the internal pterygoid; and the gland descended over the hyoid bone and the thyrohyoid muscle (whose nerve it completely covered) to a point 2¾ inches below the angle. The submental artery, lying well below the mandible, came off the facial only after this had crossed the gland. The intermediate tendon of digastric was not covered by the gland, which, in ligature of the second part of the lingual artery, would not have been in the way, although it could have been retracted backward instead of upward.

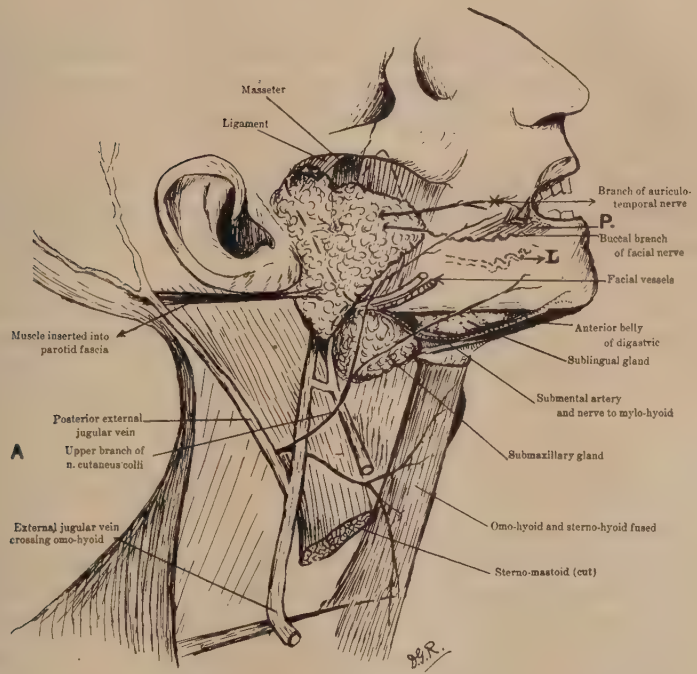

Fig. 1 Drawing constructed from two specimens (male and female adults). The tendon of omohyoid is prolonged into an intersection on the deep aspect of sternohyoid, and along with this there is a blending together of these two muscles. This occurred only in four in 373 bodies (Turner). The 'posterior external jugular' vein drains most of the blood from the occipital veins. *L* indicates the flexuous course of the lingual nerve in relation to genioglossus. The buccal branch of the facial nerve is also flexuous in front of masseter. This prevents stretching during the swelling of the cheeks. More attention might also be drawn to the fact that *A* normally sends branches to the face. The auriculotemporal nerve may supply a large part of the skin of the cheek. It here sent a strong communication to the buccal branch of the facial and a very strong independent branch extending to the angle of the mouth. At first such a nerve may be mistaken for a branch of the facial. In several specimens all the fasciculi of platysma (*P*), behind those attached to the mandible, passed deep to triangularis to the skin of the outer part of the lower lip. None were carried to their insertion behind triangularis as figured by Quain, Morris, Poirier, Sappey, etc. Sometimes triangularis has a small deep head of origin. In the male the subclavian artery lay behind the subclavian vein in front of scalenus anterior on both sides. In lectures the fore part of the neck is best 'built up' upon the parts in the middle line in front of the vertebral column as a 'foundation-stone.' A discussion on the contents of the so-called 'anterior triangle' only confuses the student, but can be undertaken critically later on.

osseous surface, and, more rarely, as Poirier has stated, viz., "logé dans l'angle dièdre formé par la face interne de la mâchoire et la face externe du mylo-hyoïdien" where it "affecte un rapport important avec le bord supérieur de la glande sous-maxillaire."

In the three situations last named it would readily be displaced downward along with the submaxillary gland, to which of course it is also tied by branches, by pressure exerted from above.

It is possible the mode of termination of the facial (anterior facial) vein, which crossed the submaxillary gland under cover of the overlapping lower part of the parotid, was correlated with the displacement. It passed entirely into the vein (fig. 1), here quite large, which follows the anterior border of the sternomastoid muscle and normally connects the facial with the lower part of the anterior jugular vein.

"Petits prolongements qui traversent les interstices qui séparent les faisceaux du mylo-hyoïdien" have been noted (Poirier's Anatomy, 1905, vol. 4, p. 695).[2] But what I have described, especially since bilateral and symmetrical and with displacement of the submaxillary gland, must be very rare and seems well worthy of being placed on record.

For other observations, see the legend to figure 1.

[2] See also M. Moustin, "Pathogénie de la grenouillette sus-hyoïdienne," Gazette des Hôpitaux, 1897, no. 53. I have been unable to get this publication.

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

FORTY-SECOND SESSION

Yale University School of Medicine, New Haven, Connecticut, April 1, 2, and 3, 1926

Thursday, April 1, 1926

10.00 a.m. The Forty-second Session of the American Association of Anatomists was called to order by Vice-President Arthur W. Meyer, who appointed the following committees:

Committee on Nominations for 1926: Professor E. R. Clark, Chairman, Professor Edgar Allen, and Professor V. E. Emmel.

Auditing Committee: Professor E. A. Boyden, Chairman, and Professor John C. Donaldson.

The remainder of the morning session was devoted to the presentation of scientific papers.

Friday, April 2, 1926

12 noon. Association Business Meeting, with Vice-President Arthur W. Meyer presiding. The Secretary reported that the minutes for the Forty-first Session were printed in full in The Anatomical Record, volume 30, no. 1, pp. 67–74, inclusive. On motion, seconded and carried, the minutes of the Forty-first Session were approved by the Association as printed in The Anatomical Record.

Report of the Auditing Committee: Professor Edward A. Boyden reported for the Auditing Committee as follows: "The undersigned have examined the accounts of the Secretary-Treasurer for the period of December 31, 1924, to December 31, 1925, and have found them to be correct."

[Signed] Edward A. Boyden,
John C. Donaldson.

On motion, the report of the Auditing Committee was accepted and adopted.

Report of the Treasurer: The Treasurer made the following report for the year 1925:

Balance on hand, December 31, 1924 (last audit of accounts)	$376.31	
Receipts from dues, 1925	905.48	
Interest—6/29/25, $2.83; 12/30/25, $8.22	11.05	
Total credits		$1,292.84
Expenditures for 1925:		
Postage	$55.00	
Printing and stationery	89.75	
Expenses of Secretary (Cleveland Meeting)	50.26	
Wistar Institute (abstracts, Anat. Rec.)	38.40	
Secretarial services	49.50	
Union Amer. Biol. Soc. (biological abstracts)	442.00	
Total expenditures		724.91
Balance on hand, December 31, 1925		$567.93

Deposited in name of American Association of Anatomists, in Alexander Brown & Sons, Baltimore, Maryland.

On motion, the report of the Treasurer was accepted.

Election of Officers. Professor C. R. Stockard, Chairman of the Committee on Nominations for 1925, placed before the Association the following nominations for office:

For President: Professor George L. Streeter, Department of Embryology, Carnegie Institution of Washington.

For Vice-President: Professor Stephen W. Ranson, Washington University.

For Secretary-Treasurer: Professor Lewis H. Weed, Johns Hopkins University.

For members of the Executive Committee with term expiring 1929: Professor George W. Corner, University of Rochester; Professor Edmund V. Cowdry, Rockefeller Institute for Medical Research.

On motion, seconded and carried, the Secretary was instructed to cast a ballot for the election of the above named.

New Members. The Secretary presented the following names recommended by the Executive Committee for election to membership in the Association:

Arataki, Minoru, *Director Arataki Hospital, Asahigawa, Japan.*

Burns, B. I., M.D., Professor of Anatomy, *University of Utah, Salt Lake City, Utah.*

Cairney, John, M.B., Ch.B., M.D., Associate Professor of Anatomy, *Department of Anatomy, University of Otago, Dunedin, New Zealand.*

Coman, Francis Dana, A.B., A.M., M.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

Copenhaver, Wilfred M., A.B., Ph.D., Instructor in Anatomy, *University of Rochester, School of Medicine, Rochester, N. Y.*

Engle, Earl Theron, A.B., A.M., Ph.D., Instructor, *Department of Anatomy, Stanford University, California.*

Gurdjian, Elisha Stephens, A.B., M.S., M.D., Instructor in Anatomy, *Department of Anatomy, University of Michigan, Ann Arbor, Michigan.*

Hargitt, George T., Professor of Zoölogy, *Syracuse University, Syracuse, N. Y.*

Hogue, Mary J., Ph.D., Instructor in Normal Histology, *University of Pennsylvania Medical School, Philadelphia, Pa.*

Morgan, Lawrence Onis, A.B., A.M., Instructor in Anatomy, *Cornell University Medical College, Ithaca, N. Y.*

Morton, Dudley Joy, M.D., Instructor in Orthopedics, *Yale University School of Medicine, New Haven, Conn.*

Scott, Gordon H., Ph.D., Assistant Professor of Anatomy, *Loyola University School of Medicine, Chicago, Ill.*

Scott, J. Terrell, A.B., A.M., M.D., Instructor in Anatomy, *University of Colorado Medical School, Denver, Colo.*

Tsai, Chiao, Ph.D., Professor of Comparative Psychology, *Fuh Tan University Kiangwan Shanghai, China.*

Wieman, H. L., Ph.D., Professor of Zoölogy, *University of Cincinnati, Cincinnati, Ohio.*

On motion, seconded and carried, all candidates proposed by the Executive Committee were declared duly elected.

Dropped Members. The Secretary then announced the following names as dropped from the list of members on account of non-payment of dues for the past two years:

Dr. J. T. Carter, London, England.
Dr. J. D. Craig, Albany, N. Y.
Dr. E. Fawcett, Bristol, England.
Dr. J. B. Gatenby, Dublin, Ireland.
Dr. A. Hopewell-Smith, Philadelphia, Pa.
Dr. M. Inouye, Tokyo, Japan.
Dr. R. Lefholz, Oak Grove, Mo.
Dr. E. M. Watson, Buffalo, N. Y.

Death of Members. The Secretary then announced, on behalf of the Executive Committee, that during the past year the Association had suffered great loss in the death of the following members:

Dr. G. A. Bates, Boston, Mass.
Dr. F. W. Carpenter, Hartford, Conn.
Dr. A. C. Eycleshymer, Chicago, Ill.
Dr. D. G. Methany, Philadelphia, Pa.
Dr. S. Simpson, Ithaca, N. Y.
Dr. O. Van der Stricht, Ghent, Belgium.

Resignation of Members. The Secretary reported that the following members had resigned from the Association during 1925:

Dr. Edwin S. Goodrich, Oxford, England.
Dr. F. M. Nicholson, New York City.
Dr. Randolph West, New York City.

Place of Next Meeting. It was announced that the Executive Committee had postponed decision regarding the place of meeting of the Forty-third Annual Session of the Association. The possibility of the Association meeting with the Federation of American Societies for Experimental Biology was commented upon.

Dues for the Year 1926. The Executive Committee recommended that the dues for the year 1926 be set at $1.00 per member. This recommendation of the Executive Committee was adopted by the Association.

Member of Journal Committee. The Executive Committee nominated the following members of the Association, one of whom was to be elected to serve on the Journal Committee as organized at the Thirty-seventh Session of the Association: Professor H. M. Evans, Professor S. W. Ranson, and Professor I. Hardesty.

As a result of the election, Professor S. W. Ranson was chosen to serve for five years.

Report of the Journal Committee. Professor H. M. Evans reported, on behalf of the Journal Committee, that the condition of publication in the various anatomical journals was becoming more and more satisfactory. The Committee has decided to request each year from The Wistar Institute of Anatomy and Biology information regarding finances and other matters relating to publication.

On motion, the report of the Committee was accepted.

Report of Committee to Investigate the Status of Anatomy. In the absence of Professor H. McE. Knower, Chairman of the Committee, the Secretary read in full the report submitted by Professor Knower. This communication related to: 1) Changes in requirements for admission to medical schools adopted by the Association of American Medical Colleges, permitting partial substitution of Psychology or Sociology for the premedical requirements in Biology. 2) The desirability of introduction of elementary embryology as part of the premedical requirements in biology.

After discussion, the Association, on motion, voted to lay the report of the Committee on the table.

On motion, the business session then adjourned.

Saturday, April 3, 1926

12.45 p.m. Concluding business session. A short business meeting followed the morning scientific session. The Secretary reported that there was no unfinished business.

Professor Harold D. Senior presented the following resolution:

Resolved: That, in concluding one of its most enjoyable and interesting meetings, the American Association of Anatomists wishes hereby to tender a resolution of thanks and appreciation for the kind hospitality extended to it by the Officers of Yale University and in particular by those of the Medical Department.

To the Members of the Anatomical Staff and to the Committee on Arrangements, the Association is specially indebted for the manner in which the admirable facilities of the Sterling Hall of Medicine have been placed at its disposal.

Moved, seconded, and unanimously voted.

On motion, the Session adjourned.

LEWIS H. WEED,
Secretary of the Forty-second Session of the American Association of Anatomists.

AMERICAN ASSOCIATION OF ANATOMISTS

OFFICERS AND LIST OF MEMBERS

Officers

President.....George L. Streeter
Vice-President.....Stephen W. Ranson
Secretary-Treasurer.....Lewis H. Weed

Executive Committee

For term expiring 1926.....H. McE. Knower, Richard E. Scammon
For term expiring 1927.....Charles R. Stockard, Clarence M. Jackson
For term expiring 1928.....J. Parsons Schaeffer, T. Wingate Todd
For term expiring 1929.....George W. Corner, Edmund V. Cowdry

Members of Journal Committee and Expiration of Term

Frederic T. Lewis (1927)
Charles R. Bardeen (1928)
Clarence M. Jackson (1929)
Harvey E. Jordan (1930)
Stephen W. Ranson (1931)

Delegate to the Council of A. A. A. S.

Harold D. Senior

Representative to the National Research Council

H. H. Donaldson

Committee on Nominations for 1926

Eliot R. Clark, *Chairman*
Edgar Allen
Victor E. Emmel

Honorary Members

S. Ramón y Cajal.....*Madrid, Spain*
Camille Golgi.....*Pavia, Italy*
A. Nicolas.....*Paris, France*
Wilhelm Roux.....*Halle, Germany*

MEMBERS

ABBOTT, MAUDE E., A.B., C.M., M.D., F.R.C.P.& S., Assistant Professor of Research Medicine, McGill University, *836 University St., Montreal, Canada.*

DE ABERLE, (MRS.) SOPHIE B., A.B., A.M., *Stanford University, Palo Alto, Calif.*

ADAMS, A. ELIZABETH, A.B., A.M., Ph.D., Associate Professor of Zoölogy, *Mount Holyoke College, Mount Holyoke, Mass.*

ADAMS, L. A., A.B., A.M., Ph.D., Assistant Professor of Zoölogy, *University of Illinois, 605 West Indiana St., Urbana, Ill.*

ADDISON, WILLIAM HENRY FITZGERALD, B.A., M.D., Professor of Normal Histology and Embryology, *School of Medicine, University of Pennsylvania, Philadelphia, Pa.*

ADELMANN, HOWARD B., B.A., M.A., Ph.D., Instructor in Histology and Embryology, *Cornell University, Stimson Hall, Ithaca, N. Y.*

ALFORD, LELAND BARTON, A.B., M.D., Associate in Clinical Neurology, Washington University School of Medicine, *Humboldt Building, St. Louis, Mo.*

ALLEN, BENNET MILLS, Ph.D., Professor of Zoölogy, University of California (Southern Branch), *909 Micheltorena St., Los Angeles, Calif.*

ALLEN, EDGAR, A.M., Ph.D., Professor of Anatomy, *University of Missouri, Columbia, Mo.*

ALLEN, EZRA, A.M., Ph.D., Professor of Biology, *Ursinus College, Collegeville, Pa.*

ALLEN, WILLIAM F., A.M., Ph.D., Professor of Anatomy, *University of Oregon Medical School, Portland, Oregon.*

ALLIS, EDWARD PHELPS, JR., C.E., M.D., LL.D., *Palais de Carnoles, Menton (A.M.), France.*

ANSON, BARRY J., A.B., A.M., Medical Fellow of the National Research Council and Fellow in Histology, *Harvard University Medical School, Boston, Mass.*

APPLEBY, J. I., A.B., M.B., A.M., M.D., *105½ West Main St., Bellevue, Ohio.*

ARAI, HAYATO, M.D., Chief Gynecologist, *Sapporo Hospital, Sapporo, Japan.*

ARATAKI, MINORU, *Director Arataki Hospital, Asahigawa, Japan.*

AREY, LESLIE B., Ph.D., Robert Laughlin Rea Professor of Anatomy, and Chairman of the Division of Anatomy, Northwestern University Medical School, *2421 Dearborn St., Chicago, Ill.*

ATTERBURY, RUTH RAND, A.M., Ph.D., *144 Overlook Ave., Great Neck, L. I.*

ATWELL, WAYNE JASON, A.M., Ph.D., Professor of Anatomy, University of Buffalo Medical College, *24 High St., Buffalo, N. Y.*

BADERTSCHER, JACOB A., Ph.D., Professor of Anatomy, Indiana University School of Medicine, *312 South Fess Ave., Bloomington, Ind.*

BAGLEY, CHARLES, JR., M.D., *The Latrobe Apts., Baltimore, Md.*

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BUILDING PLANS FOR THE ANATOMICAL DEPARTMENT, WITH A DISCUSSION OF THEIR RELATIONS TO CLASS TEACHING AND TO THE DEVELOPMENT OF THE INDIVIDUAL STUDENT

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The following account of general principles which should influence the planning of a building for a department of anatomy is written because of the experiences of the writer in a number of conferences since planning the anatomical wing of the Medical College of the University of Cincinnati, the plans of which have not before been discussed in print.

The writer's plans are, in the first place, the result of a long teaching experience in the Johns Hopkins Medical School in Professor Mall's laboratory, which incorporated many desirable features at the time it was built, in a well-thought-out plan adapted from the best European traditions. In addition, coping with mistakes or inadequate arrangements in various laboratories, together with an examination of published plans, also help us to formulate some definite principles of general value.

Professor Mall described his building in an important paper in the Johns Hopkins Hospital Bulletin (vol. 7, '96), thirty years ago. This paper contains much discussion and information still of great interest and is a fundamental outline. Since that time, new methods of construction and modern service facilities have made it possible to secure better results in a number of things, while following the lines then so well laid down. Hence the writer was able to profit considerably by personal examination of the principal laboratories in the

country while on a trip of investigation preliminary to planning the anatomical wing of the medical building of the University of Cincinnati. The then new laboratories at Minneapolis and St. Louis were especially instructive. The Cincinnati Department was built in 1916, strictly in accord with my plans, which have proved acceptable, even though the number of students cared for has exceeded the fifty set as the limit for the best use of its class rooms.

Overcrowding is to be regretted, for unfortunately it is very apt to inhibit the staff from undertaking valuable lines of development herein outlined further on, for which arrangements were built in, and the time of staff and space in the building must be devoted chiefly to handling large classes instead of to work of a higher order.

More recently, several newer anatomical buildings have been planned or already erected with excellent features, so there are now quite a variety of alternatives to examine in a number of places for the working out of details.[1]

It is not the object of the writer to urge duplication of the Cincinnati plans as against any other, but to endeavor to present certain essentials which should be provided in any plan, because of the observation that when plans are considered, details or special features are too often brought out rather than fundamental factors. Experience has certainly furnished some reliable basal principles, and loss of efficiency in important interrelations and conveniences will surely follow if these are overlooked.

Another reason for the paper is the importance of calling attention to the need of more space and facilities for a different type of work to follow the first dissection and for graduates. The cutting down of time for the so-called 'required' course in anatomy in this country has nearly robbed the students of opportunity for study in several im-

[1] Every one interested in anatomical and other medical laboratories will welcome the valuable series of illustrated articles published by Dr. R. M. Pearce, Director, Division of Medical Education of the Rockefeller Foundation, entitled Methods and Problems of Medical Education—1924, 1925, etc.

portant aspects of the subject. Dissection is now too often treated as a mere analysis ending before any adequate constructive conception of the body is gained. Repeated dissection takes much time without adding any particularly new point of view. Probably the better method might be to make dissection with its valuable training in methods of objective study and analysis, the main feature of the introductory course of 'required' anatomy; and then arrange to follow this up by all promising methods which shall help build up a synthetic knowledge of the body and bring about correlation with other subjects of the curriculum.

New facilities for such work are needed in special 'study rooms' where the student may continue anatomical studies throughout his medical course; not only in a part of his first year. These arrangements are considered further on.

Perhaps such a revival and extension of the 'study rooms,' the main features of which for his time were so well pictured by Professor Mall, will now help to correct the tendency to reduce anatomical teaching to a mere preliminary examination of the body. The individual student will here learn to make an integration of all the various aspects and interrelations of this subject, and exceptionally may even be encouraged to venture into experimental research.

In planning a building, then, the anatomical department should be thought of as a mechanism which will be most useful if built all through in such a manner that every important subdivision has the best location and relations for good reciprocal functioning. Failure to realize this with separation of parts, as of histology from the rest, has seriously affected departments in some of our schools.

While it is of primary importance that the director who is to represent the leadership in policies which the building is aimed to carry out should have most to say about the plans, still a number of expensive modern experiments in building have given us certain fundamentals which any director should welcome as furnishing commonly accepted essentials of arrangement. So the general requirements of the ana-

tomical department can be laid down, at once and economically, and specific adjustments to particular needs made later in plans which are sufficiently plastic.

The following considerations should influence the preliminary plans to insure an effective organic whole:

GENERAL

1. Sufficient separation from other departments to permit of future outbuilding or extensions and to secure freedom from disturbances with certain general advantages for best functioning. Wide-open communications with other departments, or the incorporating of rooms and service arrangements belonging to a different department, will be disturbing and uneconomical in the building and its use.

2. Good drainage with sufficiently large reception sewers for excess waste water should be assured.

3. Ample provision for heating, refrigeration of some rooms, delivering power, steam, direct current, etc., should be insured by direct relations to a power-house.

4. Decision as to the number of students to be cared for determines the requirements for space; but when certain fixed needs are built in at the start for the first fifty students, extensions to care for an increase can be made at relatively small cost.

SPECIAL

Gross anatomy: A. Dissection, analysis. B. Reconstruction, synthesis, correlation

The provision of sufficient space for gross anatomy as here understood, on one floor, will be found to give ample room for other classes and work on the remaining floors.

Third floor. A. Dissection. General dissecting rooms. Each dissecting table with the necessary room for students around it and passageway between adjoining tables will require about 10 ft. by 10 ft. This includes allowance in these rooms for wash sinks and window tables on the walls. If we put four men to each table, forty-eight students will

accordingly require a room 30 ft. by 40 ft. (1200 sq.ft.). This is an excellent size for handling a class of fifty as a whole, and no larger room is advisable. Two such rooms with doors or rows of pillars between, totaling about 2400 sq.ft., will give the best arrangement for one hundred students dissecting in class, if so large a number is to be handled. With glass partitions between the rooms, the whole class can be kept in view without undue noise or confusion. No pillars should support the ceiling inside the areas just given, the entire floor space being left free for movable tables, cases, and stools, with the view unobstructed. These large rooms will be about 12 ft. high.

If other groups of students must dissect, they should have separate quarters or use these rooms at a different time of the year when free. As is pointed out below, there are important reasons to insist on the location of dissecting rooms on the North side of the building.

B. Synthesis, reconstruction, correlation. 'Study rooms.' In addition, gross anatomy should be thought of as a synthesis as well as a dissection, as has been said, and hence we must set down here the arrangements to be made for such work. There should be in close proximity (this is important) to the large dissecting rooms considerable extra space, generous space, divided by light partitions, as judged best, into a series of rooms not only for special dissecting, but for work with the latest methods of correlational anatomy, always with functional stress, through the combined study of a variety of specimens. There should be one large general 'study room,' and besides a number of smaller ones for work in the following lines: 1) sections of the body; 2) x-ray and fluoroscopic examinations, with special developing rooms; 3) careful physical examination of the living body in part with special instruments as preliminary to diagnosis; 4) variations and embryonic relations; 5) special dissections, models, etc.; 6) possibly a moving-picture room; 7) specimens from experiments and of special physiologic interest. An attendant preparator should be constantly on hand here to make available the specimens accumulating from work on this floor.

In these rooms individual students will find ample opportunity to follow up suggested lines of inquiry often leading to continued research.

These rooms with enough space added for storage, a dissecting-gown room, preparation room, small emergency toilet, janitor's closet, halls, surgical anatomy laboratory, etc., will add to this floor an area of more than four times the size of the large dissecting room already estimated at about 1200 sq.ft. for fifty students.

Professor Mall advocated a series of small study rooms in which groups not only dissected, but at the same time learned from the beginning much accessory anatomy, with the help of models, sections, etc.[2]

This deliberate, truly scientific method of learning would certainly be the most desirable one for all students, but takes far more time than we are now allowed for beginners. Its general adoption would also now be prohibited by the present schedule, since these students are usually handled in classes and their required time is limited to less than one-half of that at Professor Mall's disposal; so there hardly remains time to satisfactorily complete a dissection before they must turn to other subjects. Under these conditions, the large dissecting rooms here suggested seem preferable for the beginning class, where a number of bodies together present the advantage of ready comparisons from one to another. Variations, anomalies, and even abnormal features can here be shown and discussed with the entire class. In addition, we may now have in the department, near by, x-ray and fluoroscope rooms and other arrangements for examinations of the living. With the rich material of the dissecting rooms in adjoining quarters, the opportunity of a better 'study room' is now created of constant use to all classes of students and staff. Graduates will always find an ample supply of specimens here to satisfy their need to look up anatomical relations, and special work they accomplish can be added to the collection.

[2] See also Bardeen in Pearce's Method's and Problems of Medical Education, series I, Rockefeller Foundation, 1924.

The entire floor space on the third floor allotted to dissection, correlation 'study rooms,' x-ray, hall-ways, etc., calculated thus, would total about 7200 sq.ft. for fifty students—now perhaps an average class. This would constitute a floor in a building 120 ft. by 60 ft. Only a relatively small extension would be required for one hundred students, who would demand an area of 160 ft. by 60 ft., or 9600 sq.ft., for this floor.

Strong arguments are being advanced for the policy of admitting larger freshman classes. If this is to be done, the college authorities should realize that it affects first and most seriously the anatomical department, and should see to it that adequate provision, generous provision, is made for building space and equipment to avoid crowding out of the higher and most constructive functions of the department.

Naturally, this department has to do a maximum of training and selective work, through personal laboratory contacts in both gross and microscopic courses. For this it should be granted an especially large and capable staff, with a budget sufficient to care for the double burden of the two major courses for beginners, as well as for the development of more advanced work. Before increasing the burden by taking more students, the anatomists in most colleges should be given decidedly more help to solve their present peculiarly difficult problems.

Of course, the 'study rooms' could also be used at certain times by other groups than the regular students, but they should be open and their facilities available through the help of an attendant most of the time. No allowance is made here for a separate lecture room for the floor, though it will be advisable to have access to one conveniently located, and probably a minimum space has been allotted.

The installation of electricity, gas, water, and facilities for work in these rooms is a special problem, and evidently a service elevator from the basement should be available for this floor as for others. The large general 'study room,' at

least, should have northern exposure to protect the material kept here from drying.

It is becoming more and more evident that in some way the individual student must be discovered in the crowd and given new opportunities, under specially favorable conditions, for development in the course of his training. Well-equipped 'study rooms' offer an exceptional chance for this by the use of improved methods.

In this way the constructive side of the department of anatomy will be given a chance to assert itself, through a new emphasis on such individual advanced work, while the elementary routine for the class will be relegated to its proper position of subordination. Some of this can be accomplished by encouraging student electives, until it becomes possible to give more individuals separate facilities and responsibilities, here, for independent or semi-independent work. This will require changes in the schedule as well as room for work. As far as we know, Yale University is the first, in this country, to break away from the class method of teaching in the first two years; though, as we have pointed out, various anatomists had already done much to encourage an individual method of work for their students.

In the meantime a special professor with assistants may be in charge of groups of students in the 'study rooms,' and clinicians should be invited to help out, here with work on the living subject. Following the class sessions, the students will find ample material for personal studies.

Of course, these departures may mean more work for the staff and a larger staff, but such work will be constructive and stimulating as compared with the deadening effect of trying to teach oversized classes of unoriented freshmen, and additions to the staff are certainly justified to develop work of this character.

Undoubtedly, in most laboratories many individual students have profited by such studies as are here advocated for a regular system throughout each man's medical course. Professor Mall pointed out the value of the method, and it can

be seen in operation, here and there, as a not unusual practice, at least in the first two years.

A number of departments have maintained 'study-room' courses as chiefly reconstruction from sections, either with sophomores or in the spring with freshmen as soon as they shall have finished most of their dissection. This is a valuable supplement to the usual somewhat hurried and incomplete introductory exploration, but has not satisfied the need for an effective stimulus to induce students to keep up their anatomy throughout their medical course.

A broader course is needed, with a deliberate effort to relate anatomy more closely to the other subjects of the curriculum and to establish a habit in each student of resorting to the study rooms constantly for all sorts of clinical anatomy. Such courses are being discussed and tried in various forms.

One was inaugurated by me in Cincinnati in the spring of 1921 by inviting the cooperation of the clinical staff. We found their views and leadership in special fields most useful while they relied on the anatomist for their ground work. The class examined each other and even went over the physical signs of some hospital cases, while related specimens were kept out for the students to study, between times, in the 'study rooms' where our facilities were always available. This course has been continued ever since with success as a study of the anatomical basis of physical diagnosis, and seems to be helpful to the students and to bring the clinical departments into closer touch with anatomical foundations of their work.[3]

To develop the best possibilities of this work, it will be necessary to extend and thoroughly equip the 'study rooms' and provide sufficient increases to the regular staff and technical assistance. In addition, the encouragement and help of the clinicians are essential.

These courses, however, should be regarded as only stepping-stones to a freer system, where each individual from

[3] See also Malone on previous work in correlational anatomy at the University of Cincinnati. Anatomical Record, vol. 8, 1914.

the beginning to the end of his college work seeks the 'study rooms' for the comparisons and correlations which will make his clinical work more exact and reliable. The graduate will also appreciate the ease with which he can consult special anatomy and the saving of his time.

Interrelations of storage and preparation of material in basement, with arrangements for work on third floor and roof. Whatever the arrangements of the dissecting rooms and 'study rooms' are to be, these should be connected directly with a separated suite in the basement where bodies are prepared and stored. This connection should be a shaft sealed off from the rest of the building and enclosing a small electric lift to carry bodies (three at a time upright), as well as to carry sections, etc., from the vault in the basement to the gross study suites on the third floor and to the roof. This shaft should only open into the basement vault, on the one hand, and the third floor and roof suite, on the other. The general service elevator of the building should be entirely apart from such suites and from the part of the basement concerned with cadavers.

There should be also convenient access from the dissecting rooms to a suite on the roof for the preparation of skeletons, maceration of parts, and incineration; also one or more rooms for animals.

To keep the building as free from odors and unpleasant disturbances from the dissecting and similar work and to insure a maximum privacy from outside observation or interference, the third floor will be found to be the best place for gross anatomy.

As has been said, the large class laboratories and the storage vault should be located along the north wall. This will secure the greatest economy in the care of specimens and avoid the drying of dissections and wet sections, as well as give good lighting and the greatest amount of comfort for the workers. The large dissecting rooms and general 'study rooms' should also be placed on the north side. Skylights should be prohibited from the same need of cutting out the

hot sun's rays. Instead, 4 ft. of air space should be left between the roof and ceiling of these rooms for best results. Prism-glass window panes will reinforce the general lighting, and electric lights, properly placed, give ample special illumination.

Basement. Reception of material, special vault, embalming suite, shop. The rooms in the basement devoted to embalming, preparation of bodies, making sections, etc., should be closed off and screened from the rest of the building; while the bodies should be kept in tanks in a long vault extending along most of the north side, thus protected from exposure to the sun's direct heat. This vault should be 8 ft. high inside and built within the basement, on the north side, with its top well below the first floor, and its one open end communicating with a reception room well aerated through screened north windows and containing the beginning of the private elevator shaft for the bodies, also facilities for cutting sections (special current needed here for power). Fresh air should be drawn into this vault from the reception room, through the vault, and upward to the outside through a special ventilating duct with a fan at the outer end. This is a simple system, readily installed, and should be separated from the ventilation of the rest of the building. There will be ample space for the storage of bodies in this vault. The floor should be water-proofed and drained into the sewers.

This completely isolated arrangement with separate ventilation in the plans of the Cincinnati department is, I believe, original.

The above-described suite should be walled off to itself, and service pipes, hot-air ducts, wires, etc., for the rest of the basement and building should be carried along over the top and outside of the vault.

Experience has proved to most anatomists who have tested the facts that large expensive refrigerators are unnecessary. Bodies are best stored, when once well embalmed, in tanks of carbolic solution (concrete tanks 6 ft. by 4½ ft., 3 ft. deep inside measurement, each to hold ten to twelve bodies). A

relatively small refrigerator, say 7 ft. by 7 ft. by 7 ft. inside measurement and capable of regulation from zero, with a cooled ante-chamber opening into the vault which encloses the tanks, is, however, much needed. Arrangements should be made at the rear of the building for an entrance to permit the undertaker to deliver bodies from the automobile under cover near the embalming room. An overhead track for moving bodies through the vault, to and from tanks and lift is not only convenient, but economical in service.

The basement should include, in addition to the vault suite, general storerooms, a special dark storeroom with no windows, one or more rooms for animals opening out on the adjoining grounds, a room for containers and water tables to hold frogs and other amphibia, and a good room for the embalmer with private toilet and shower. A small shop should be connected directly upward with the research floor (first floor) by a private staircase. This shop should have power, steam, electric outlets, gas, air pressure, water and a hood, and is an essential for the department in making apparatus, models, cages, special adjustments of all sorts as well as small repairs. It should be included even if there is a general shop elsewhere, for it represents many economies.

The separation of the vault, its flooring, the tanks, plumbing (best exposed everywhere in the building), ventilation, and drainage, and the handling of the pipes and hot-air ducts of the basement are special and very important problems.

Connections of gross storerooms, shop, etc., with research and preparation rooms. The main general storerooms should be located in the basement, and a very great convenience running up from the shop here is a private staircase and dumbwaiter connecting with the technique room on the first floor, and further up to the class preparation room on the second floor, which stands in vertical line above the shop. This is a most useful association of service rooms.

Second floor. Microscopic courses and photography. A. General, class suites. The second floor must have facilities for teaching histology, embryology, neurology, and other

microscopic subjects. These are the elementary introductory courses taught to large classes. A good-sized preparation room for general technique with a storeroom, cold box, hood, etc., adjoining the class rooms is needed at the end of this floor to the east, where it can be useful to all rooms of the entire floor. Photographic facilities also belong here in a communicating suite with special current for projection, moving pictures, etc. The desirable connections of these suites with the research floor have been already described. If possible, the photographic rooms should have north light; indeed, a northern exposure seems essential for all these large classes in microscopic work. A woman's toilet and janitor's closet for supplies are needed.

To accommodate fifty men, it may be better to subdivide the space for class rooms into three rooms. A larger one for twenty-four students may be used as a combined lecture room and laboratory with four at a table for this section of the class. It is fitted up with demonstration table at one end, lanterns, chart holders, etc., for use as a lecture room for the entire class as occasion demands. Two smaller rooms in series will be laboratories for sixteen each, four at a table. An instructor to each sixteen to twenty students is the correct allotment for microscopic work with beginners.

If other classes of students are to use these rooms at different times, separate sets of lockers should be provided at their desks. The arrangements of plumbing, gas, electricity, air, etc., at each table and elsewhere on this floor and on the first floor are especially important. The large class rooms should communicate directly with each other, and glass partitions between are advisable to help the instructors keep in touch with the entire class. The small supplementary laboratory rooms described in the next section for groups of two to four special workers are an important feature. They proved to be most useful in Cincinnati. In a new plan it might be better to reduce somewhat the large rooms of that plan to allow more space for these smaller ones.

A large lecture hall in adjoining quarters where anatomical material can be shown will be needed if the number of a class is much over fifty.

Of course, if a class over fifty, up to a hundred is contemplated, provision must be made for four or five large class rooms instead of three. The total space for the floor has already been determined by that given gross anatomy, and will be also ample here for all of the accommodations here planned.

B. Special, 'study-room' suites. In addition, as was planned for gross anatomy on the third floor, provision should be made on this floor for synthetic study and correlations of all microscopic and embryological facts and relations, through individual work of students with special collections, models, photography, x-rays, and personal experiments. This could become one of the strongest developments of the department. Small laboratory suites and larger 'study rooms' will be required to carry out these ideas, which involve cooperation with other departments and even with graduates for special studies. Here, again, will be a most encouraging environment for all students to begin individual research in some form.

First floor. Research. The first floor should be devoted to the staff and special research. The association of the various workers in different lines of investigation on this floor is highly important. There should be included rooms for the staff, special students, and visiting workers, with light partitions everywhere and some of these rooms connecting; a seminar room; office; library; etc., and the important general technique room, already mentioned, for work not to be carried on in private rooms. In this room will be found apparatus for common use of all on the floor, as balances, incubators, ice chest, operating table, hood, compressed air, etc. Its connections with the shop and with the class preparation rooms above by private stair and dumb-waiter have been pointed out as valuable in aiding members of the staff on the first floor and class technicians on the second floor to work together.

Much time and effort can be saved by this association, and the relations established between research and experimental microscopic class technique will prove stimulating and helpful. Each room should have generous provision of electricity, gas, water, sinks, fireproof incubator table with chairs, tables, cases, shelves, drawer cabinets, etc.

Roof. The arrangements for macerating, degreasing, and drying bones and for incinerating parts and rubbish from all over the building are best cared for on the roof, to which suite the small electric lift should be brought from the basement. A small room for making wax plates belongs here, also several for animals. For these purposes, steam, hot and cold water, electricity, gas, etc., are needed on the roof.

The details of distribution of facilities for best operation, special arrangements or equipment, doors, transoms, furniture, sinks, plumbing, fire-proof tables for incubators, special lighting, etc., are too numerous for this outline.

Many valuable special arrangements will be found worked out in our modern laboratories. Indeed, much ingenuity and skill have been displayed, and plans or photographs of these and of all sorts of equipment and conveniences may be obtained from the professors in charge. Costs vary from extravagant luxury of individual choice, when the builders had much to spend, to practical economy for general need, where the effort has been to secure essentials and save money for maintenance. Much economy is possible by adherence to a compact well-related organization of parts.

THE DEVELOPMENT OF THE SKULL OF THE TURTLE, WITH REMARKS ON FOSSIL REPTILE SKULLS

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SIXTEEN FIGURES

This paper is the report of a study undertaken at the suggestion of a distinguished paleontologist, who lamented the lack of knowledge of the development of the reptile skull. There have been of course numerous studies on the reptile skull, as the long bibliographies of Gaupp ('06) and others show. Some of Rathke's pioneer work in vertebrate embryology was done in this field. The renewed impetus to the study of the skull that Huxley's Croonian lecture gave moved W. K. Parker to investigate the reptile as thoroughly as the technical means of the time allowed. More recent studies, however, have fallen into two rather narrow groups and have concerned themselves either with the story of certain parts of the skull or with detailed descriptions of single embryonic stages. From neither group can one gather a very clear idea of just how the adult reptile skull comes into existence. The study here reported is an attempt to apply modern methods to the older and broader problem, to give a straightforward account of the development of a representative reptile skull from the precartilaginous to the adult stage.

The interest of most recent writers on the development of the reptile skull has lain in comparative embryology, and interpretative discussions have been chiefly comparisons of the form studied with other reptilian chondrocrania or with those of amphibia. In the study here presented, the author

has sought for any light the developmental story might throw upon the adult reptile skull of living and fossil forms. At the end of this paper there is added a short discussion of the fossil reptile skull from the standpoint of embryology.

The turtle, Chrysemys marginata, has been used for material in this work, partly because of its availability, partly because of the writer's previous experience with it, and partly because of the possible value its embryology might have for students of fossil forms. Despite its specialized carapace, the turtle still remains our best living example of a long-departed animal world. It is much nearer the generalized Permian vertebrates than any living form easily available for embryological study.

The story of the turtle skull falls naturally into two parts—the development of the chondrocranium and the replacement of the cartilaginous skull by the bony one.

THE CHONDROCRANIUM

In Chrysemys the cartilaginous skull reaches its greatest development in a 19–mm. embryo (figs. 11 to 13). The brain is then enclosed in an incomplete cartilaginous shell. The parts of the brain behind the hypophysis lie in a box, the floor of which is formed of cartilage laid down on either side of the notochord and of remains of the hypoglossal vertebrae, the sides by the otic capsules, and the roof by an extension of the last to form a tectum. The parts of the brain anterior to the hypophysis lie in a scalepan-shaped structure known as the planum supraseptale. The planum is supported by an interorbital septum. The septum is joined to the posterior part of the skull by a Y-shaped bar, the trabecula cranii. Anteriorly, the interorbital septum continues between the olfactory capsules as the nasal septum.

The remaining parts of the chondrocranium are not concerned with the brain. Lateral to the otic capsules already mentioned are found the quadrate cartilages. Each quadrate cartilage is hollow for the reception of the middle ear. In addition, it bears an anterior pterygoid process and articu-

lates below with Meckel's cartilage of the lower jaw. The rostral end of the skull is formed by the two nasal capsules.

To follow the whole of such a complicated structure from stage to stage is a hopeless task. It is better to divide the skull up into convenient parts and to treat of each in turn. Accordingly, I shall discuss the following: 1) Prechordal region, i.e., the skull proper anterior to the hypophysis, 2) Parachordal and hypoglossal regions. 3) Otic capsules and columella auris. 4) Quadrate and Meckel's cartilage. 5) Nasal capsules.

Prechordal region

The first element of the chondrocranium anterior to the hypophysis to appear is a pair of rod-like trabeculae cranii (figs. 1 to 3). They lie close to the midline and extend from the hypophysis to the nasal sacs. In the 6-mm. stage figured, they are the only parts of the skull that are chondrified.

The second element, also present in the same stage, is an ill-defined arch of mesenchyma attached at either root to the caudal tip of a trabecula and springing across the space filled with the hypophysis. In the 6-mm. stage the arch is penetrated by the oculomotor nerve and deeply notched for the trochlear nerve. The best name for this element is 'midbrain arch' (arcus mesencephalicus). It is the 'middle trabecula' of Rathke, the 'alisphenoid-platten' of Sewertzoff, and the 'sphenolateralknorpel' of Gaupp. Rathke's name is confusing, the others assume unjustifiable associations with adult bones.

The third element has appeared in a slightly older embryo of 5.75-mm. length (figs. 4 to 6). Here one finds two lateral plates, one on either side of the forebrain, anterior to the optic nerve, and at first loosely attached to the trabeculae cranii. The two lateral plates can be called the laminae prosencephalicae.

It will be noticed, in passing, that the whole prechordal part of the skull is at first quite unconnected to the notochord or to any other part of the skull (fig. 3). In addition,

the long axis of the prechordal part makes a right angle with that of the rest of the skull, in conformity to the general outline of the brain at that time (fig. 2).

Each forebrain plate is at first attached to an independent trabecula cranii, of the sort found in the 6-mm. embryo (fig. 1). The loose connection of the plate to the trabecula becomes a more intimate one, and at the same time a center of chondrification appears between the trabeculae cranii. A general fusion of all structures ensues, and the scalepan-like support for the forebrain comes into being (fig. 4).

ABBREVIATIONS

A.m., midbrain arch
An., angular bone
A.op., ophthalmic artery
Boc., basioccipital bone
Bsp., basisphenoid bone
C.a., columella auris
Com., complementary bone
C.l.n., lateral nasal cartilage
C.p.n., paranasal cartilage
Cr.p., crista parotica
C.s., crista sellaris
C.1, 2, 3, cervical vertebrae
D., diencephalon
Dent., dental bone
Eoc., exoccipital bone
Epo., epiotic bone
Ept., epipterygoid
F.b., forebrain
F.b.p., posterior basicranial fenestra
F.end., foramen for endolymphatic duct
F.h., fenestra for hypophysis
F.m.–x., metotic fissure, foramen of vagus nerve
Fr., frontal bone
F.ve. fenestra vestibuli
F.ii–xii, foramen for the cerebral nerves
Go., gonial bone
H.b., hindbrain
Hy., hyoid cartilage
H.1, 2, 3, hypoglossal vertebrae
L.p., lamina prosencephalica
M.b., midbrain
M.c., Meckel's cartilage
Mx., maxillary bone
N.c., nasal capsule
N.s., nasal sac
O.c., otic capsule
Opo., opisthotic bone
P., palatine bone
P.a., abducens process
Par., parietal bone
P.et., epipterygoid process
P.i., interhyal process
Pl.s., planum supraseptale
Pof., postfrontal bone
P.pr., pila prootica
P.pt., pterygoid process
Prf., prefrontal bone
Prm., premaxillary bone
Pro., prootic bone
Pt., pterygoid bone
Q., quadrate cartilage and bone
Q.j., quadratojugal bone
Sa., surangular bone
S.i., interorbital septum
S.n., nasal septum
Soc., supra-occipital bone
Sq., squamosal bone
Vo., vomer
T.c., trabecula cranii
T.m., taenia marginalis
T.p., tectum posterior
Z., zygoma
ii–xii., cerebral nerves

Meanwhile the ends of the two trabeculae cranii grow forward between the nasal sacs. Beyond the cerebrum they form a single vertical plate (fig. 4) which becomes a part of the nasal septum.

Figs. 1 to 6 Wax models of the chondrocrania of embryos of Chrysemys marginata. 1 and 2, right side; 3, dorsal side from a 6-mm. embryo, Alberta Embryological Collection, series 110; 4 and 5, right side; 6, dorsal side from a 5.75-mm. embryo. A.E.C. 107. × 15.

The two forebrain laminae are next bent toward each other and partly fused together. The wide-open V is converted into a U with flaring limbs above. The fused ventral parts of the laminae together with one underlying fused trabecula make the interorbital septum (figs. 7, 9, 11), while the upper flaring limbs of the laminae form the planum supraseptale of the completed chondrocranium.

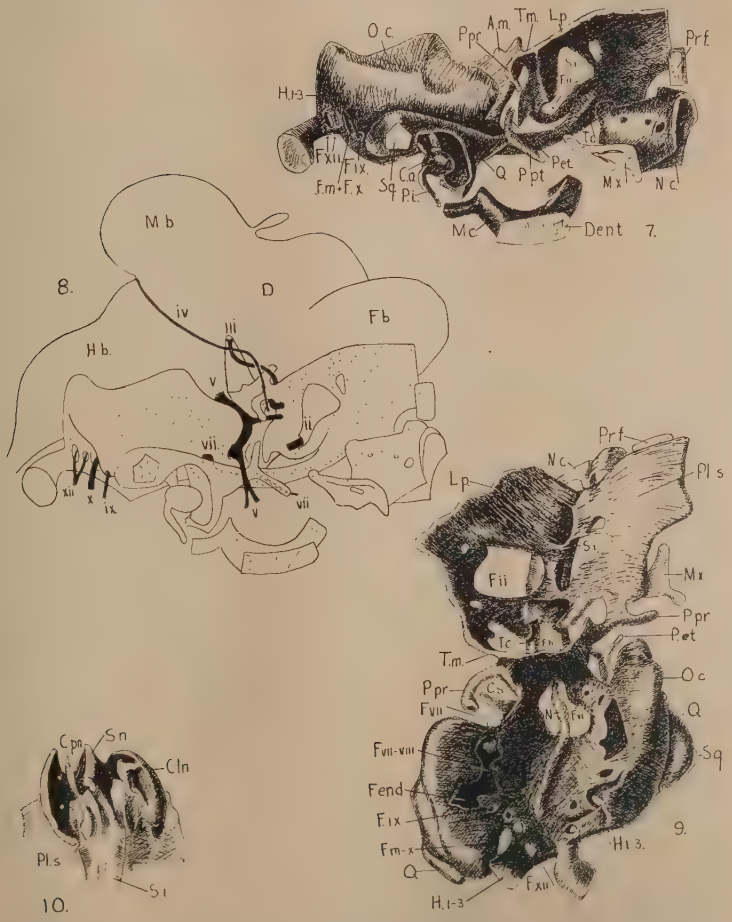
While the above changes are taking place, the midbrain arch is also growing. In the first stage modeled (fig. 1) it encloses only the oculomotor nerve. In the next model (figs. 4, 5) the arch surrounds the trochlear nerve and the ophthalmic artery, and extends in two flanges, one on either side, along the midbrain and diencephalon toward the optic nerve. The nerve then lies in a notch.

The optic notch is converted into an optic foramen by the fusion of the flange of the midbrain arch with the forebrain lamina (figs. 7, 8). A process of the forebrain lamina pushes backward beneath the nerve at the same time, between it and the trabeculae, and forms the floor of the optic foramina.

While the midbrain arch grows forward, it also extends backward and joins the tip of the notochord (fig. 4), thus forming the first connection of the prechordal region with the rest of the skull. At the same time two slender mesenchymatous processes push forward from the otic capsules. Each process is penetrated by the abducens nerve, and on that account may be called the abducens process. In the 5.75-mm. embryo modeled (fig. 6), each process is still free from the midbrain arch; shortly afterward, fusion with it takes place, and the union of the prechordal region with the rest of the skull is accomplished (compare figs. 6 and 9).

The subsequent history of the midbrain arch is one of differentiation and reduction. The sharp bend in the skull is reduced (compare figs. 4 and 7), the flange-like extensions of the arch (fig. 4) tear away from it, but remain attached to the forebrain lamina and form the posterior boundary of the optic foramina (figs. 7, 9). A persisting remnant of the connection of the flange with the parent arch forms the

taenia marginalis of other reptiles. The midbrain arch itself is reduced, the oculomotor and trochlear nerves and the ophthalmic artery regain their freedom. The arch then flattens down into a bar behind the hypophysis, the crista sellaris of



Figs. 7 to 10 Wax model of the chondrocranium and membrane bones of an 8-mm. embryo of Chrysemys marginata, A.E.C. 104. × 15. 7 and 8, right side; 9, dorsal side; 10, ventral side of nasal capsule.

Kunkel (figs. 11, 13), which continues to divide the fenestra basicranialis posterior from the foramen hypophyseos.

The early midbrain arch bears two pointed processes (fig. 6, *P.pr.*). They persist and are molded into the pilae prooticae of the mature chondrocranium (figs. 7, 9, 11). Behind the pila prootica the trigeminal nerve leaves the brain case.

Parachordal and hypoglossal regions

In the youngest stage modeled (6 mm., fig. 3), the notochord extends backward beneath the hindbrain without any special covering of precartilaginous tissue. This area between the otic capsules is gradually filled in from behind forward with unsegmented mesoderm (figs. 6, 9). The notochord lies at first above the floor plate, and then is embedded into it. Chondrification follows from no specially defined centers. When this part of the skull floor is finished, a small fenestra basicranialis remains, behind the crista sellaris (fig. 13).

The extreme posterior part of the skull floor, the hypoglossal region, has a more interesting history. Three precartilaginous hypoglossal vertebrae are formed, corresponding to the roots of the hypoglossal nerve (figs. 1 to 3). The first pair of hypoglossal arches are short and stubby, the rest differ in no wise from the cervical vertebrae that follow. The neural arches of the hypoglossal vertebrae at first lag behind those of the cervical vertebrae (fig. 6), and their bodies are fused into a common mass which joins the floor of the skull. The first hypoglossal foramen seems to be obliterated. Later, the hypoglossal arches grow rapidly and form a single big process which pushes up behind the otic capsule (figs. 7, 9) and fuses with it. A large cleft always remains between the common hypoglossal process and the otic capsule for the passage of the vagus nerve—the fissura metotica of the mature chondrocranium (figs. 9, 11).

The entire hypoglossal region is chondrified in an 8-mm. embryo. No distinct centers could be found in the bodies of the vertebrae. An ill-defined center can be distinguished in each neural arch.

Otic capsule and columella auris

The otic capsule is partly sketched in precartilage in the 6-mm. stage (figs. 1, 3). The basal and lateral portions between the facial and glossopharyngeal nerves are definite enough to be modeled. The rest of the capsule is outlined in precartilage in the next stage modeled (figs. 4, 6), and is well chondrified at 8 mm. (figs. 7, 9). No definite centers of chondrification could be made out.

In the turtle the glossopharyngeal nerve lies very close behind the otocyst, and the facial nerve similarly skirts around its anterior margin. When the precartilaginous otic capsule is laid down, it surrounds the first parts of both nerves. The glossopharyngeal runs into the large hole on the median side of the otic capsule together with the acousticofacial complex. The upper part of the vacuity is filled with the endolymphatic duct (fig. 6).

A bar of cartilage then separates the ninth from the other nerves, so that the former enters the otic capsule through a distinct foramen (fig. 9), passes through the cavity of the capsule, and emerges from a special foramen behind (fig. 7)—an arrangement that is permanent.

The intracapsular course of the facial nerve is shorter and has been questioned by Terry ('19). In the 5.75-mm. embryo (fig. 6) the combined acousticofacial trunk enters the otic capsule, and the independent course of the facial nerve does not begin until the acoustic nerve is spread out over the otocyst. The little bar of cartilage anterior to the nerve exit, the prefacial commissure, seems as much a part of the otic capsule as any other part. It seems correct, therefore, to speak of an intracapsular course for the facial nerve in turtles. The intracapsular path of the facial nerve is eventually partitioned off from the rest of the capsular cavity.

The large vacuity in the median wall of the otic capsule is gradually subdivided into several openings, one each for the glossopharyngeal nerve, endolymphatic duct, and facial nerve, and two for the acoustic nerve. One or more foramina for blood vessels also remain.

The dorsal crests of the otic capsules extend upward and form the tectum, the roof of the completed chondrocranium.

The columella auris of the turtle is simpler than in some other reptiles. When fully laid down in cartilage, it is a dumb-bell-shaped structure. The long curved shaft is divided in the middle by a constriction. The inner head or operculum fits into the foramen vestibuli, and together with its part of the shaft constitutes the columella proper. The rest of the shaft and the outer head make up what is known as the extra-columella. The outer head or insertion piece of the extra-columella bears a tapering interhyal process (fig. 11), which in a 19-mm. embryo is attached by a non-chondrified shred to the tip of Meckel's cartilage.

Much interest has been lavished on the stages leading up to the cartilaginous columella auris. The evidence of my material confirms the work of Miss L. S. Smith ('14) on the same species and harmonizes with the results of Shiino ('14) and Golby ('25) on the crocodile and alligator.

In the 6-mm. stage (fig. 1), the turtle columella, extra-columella, and interhyal process are all laid down as one piece of precartilage. The extra-columella and the interhyal process show the most advanced precartilage. The precartilage of the shaft and operculum fades out into the younger sort of which the otic capsule is made. The operculum is distinct, however, from the more or less nebulous otic capsule. The precartilage of the extracolumella is in the same stage of development as is that which forms the hyoid apparatus, a sort considerably advanced over the precartilage of the otic capsule and Meckel's cartilage.

It seems very likely that, as Miss Smith states, the interhyal process at a slightly earlier stage joined the hyoid arch. The union must have been very transient; the interhyal process of the stage modeled (fig. 4) is already turning toward Meckel's cartilage. In two other embryos of nearly the same age (A.E.C. 108, 6 mm., A.E.C. 109, 7 mm.) the interhyal process definitely joins the tip of Meckel's cartilage.

In a 7-mm. embryo a constriction appears in the shaft where it passes over the first pharyngeal pouch. The extracolumella is then marked off from the columella in the narrower sense. In an 8-mm. embryo centers of chondrification appear in the extra-columella and columella. From this time the columella auris is truly subdivided. A third center in the interhyal process is doubtful; it is imperfectly chondrified. In later stages the interhyal process dwindles (figs. 7, 11). In the adult the extracolumella remains cartilaginous; the rest is replaced by bone, a trace of which can be found in a 28.5-mm. embryo.

The old question whether the columella auris is derived from the hyoid or from the otic capsule, or both, is an unreal one. In the first place, the division of the columella is a secondary one that appears only with chondrification. The simple precartilaginous columella, like any other piece of precartilage, is laid down in situ. It is not budded off from surrounding structures. After all, the columella simply develops out of the head mesoderm as a high light does in a photographic plate, correctly fashioned and properly placed from the start.

Quadrate and Meckel's cartilage

A fragment of the quadrate and a small Meckel's cartilage are sketched in prechondrial tissue in the first stage modeled (fig. 1).

Meckel's cartilage is temporarily joined to the growing quadrate in a slightly older embryo (5.75 mm., fig. 4). It regains independence and shows traces of cartilaginous matrix in a 7-mm. embryo. At 8 mm. the right and left cartilages fuse at their anterior tips without the aid of an intercalated cartilage. The fused tips are fashioned to conform to the peculiar shape of the lower jaw and the articular facet for the quadrate is modeled out (figs. 11, 15).

The fragment of the quadrate present in the 6-mm. stage (fig. 1) expands into a definite quadrate in the next stage modeled (fig. 4). It then consists of a dorsal and posterior

mastoid portion, a rod-like articular part temporarily fused with Meckel's cartilage, and a pterygoid process. In a 7-mm. embryo cartilage matrix appears in the mastoid part and the quadratomeckelian joint is laid out. In an 8-mm. embryo (fig. 7) the mastoid part is enlarged, and an ascending epipterygoid process, homologous with the 'columella' of kinocraniate lizards, is added to the pterygoid process. The whole is now in cartilage. While the chondrocranium grows to its maximum in the 19-mm. embryo (fig. 11), the mastoid part of the quadrate is hollowed out to accommodate the middle-ear outgrowth from the first pharyngeal pouch.

The pterygoid process of the quadrate of embryos around 6 mm. in length (fig. 4) lies just above the rudimentary dental ridge of the upper jaw. The pterygoid process thus bears the same relation to the upper jaw that Meckel's cartilage does to the lower. The quadrate, its pterygoid process, and Meckel's cartilage form an oral visceral skeleton very much like that found in salmon embryos (Gaupp, '06).

Nasal capsule

The first part of the nasal skeleton to appear is the septum, the rostral part of which develops on the fused tips of the trabeculae cranii (fig. 4). When the nasal capsules appear (8 mm., fig. 7), they extend backward along the interorbital septum for some distance. The part of the interorbital septum that lies between the capsules is added to the nasal septum in the mature cartilaginous skull.

Each nasal capsule arises from two distinct cartilages which appear in the 8-mm. stage (figs. 7, 10). The smaller medial paranasal cartilage lies along the ventral margin of the nasal septum. The larger lateral cartilage forms the side and roof of the capsule. To form the roof, the lateral cartilages fuse with the dorsal margin of the nasal septum—a process already begun at this stage and completed in the next older one modeled. The floor of the nasal capsule is completed by fusion of the paranasal with the lateral cartilage, and with the ventral margin of the septum. A persisting

slit on either side of the ventral margin of the septum forms the prepalatine foramen. The tips of the two nasal cartilages are distinct at the posterior choanae long after the capsule is complete.

The general contour of the nasal capsule is governed by the nasal sacs. In the completed cartilaginous capsule (fig. 11) one can distinguish between a ventral cylindrical respiratory part, which forms a direct passage between the two choanae, and a dorsal dome-shaped olfactory portion. The line of separation is marked by a slight fold on the lateral wall of the capsule and by a parallel ridge on its inner surface. A small nodule, the pila supraglandularis of Kunkel, which is attached to the nasal septum, may separate the two parts medially.

In the mature cartilaginous nasal capsule, the upper lip of each anterior choana is deeply incised to accommodate a dorsal gland, which spreads out between the capsule and the bones that form over it.

The finished cartilaginous skull, the several parts of which have been followed in development, is found in a 19-mm. embryo (figs. 11, 12, *B*). Kunkel's ('12) admirable and exhaustive description of the mature chondrocranium of Emys is easily accessible, and no special discussion of this stage in Chrysemys need be attempted here.

DEVELOPMENT OF THE ADULT SKULL

As the chondrocranium approaches its maximum, the membrane bones appear around it, taking up at once their permanent positions and relations. The chondrocranium is no sooner finished than it degenerates. Much of it is replaced by bone developed from centers located in the cartilage matrix. This second class of cartilage bones comes into correct articulation with the membrane bones to finish off the adult skull. The remaining portions of the chondrocranium, in the turtle at least, are largely resorbed. The three steps, then, in the development of the adult skull, which can now be followed in detail, are: 1) Development of membrane bones.

2) Development of cartilage bones. 3) Degeneration of the chondrocranium.

Membrane bones

The first membrane bones are found in an 8-mm. embryo (fig. 7), in which the squamosal, prefrontal, maxillary, and dental bones appear. In the 19-mm. stage (figs. 11, 12, 13) all membrane bones are present but the parasphenoid and the doubtful membrane element of the basioccipital. The last two are found in the oldest embryo modeled (figs. 14, 15, 16).

The membrane bones of the turtle skull fall into six groups:

Group I. Dental, angular, surangular, gonial, and complementary bones. These form the greater part of the lower jaw.

Group II. Parasphenoid, palatine, pterygoid, vomer, and maxillary bones. These form the roof of the mouth, supplement the floor of the chondrocranium anterior to the hypophysis, and in the adult form the base of the skull over the same area.

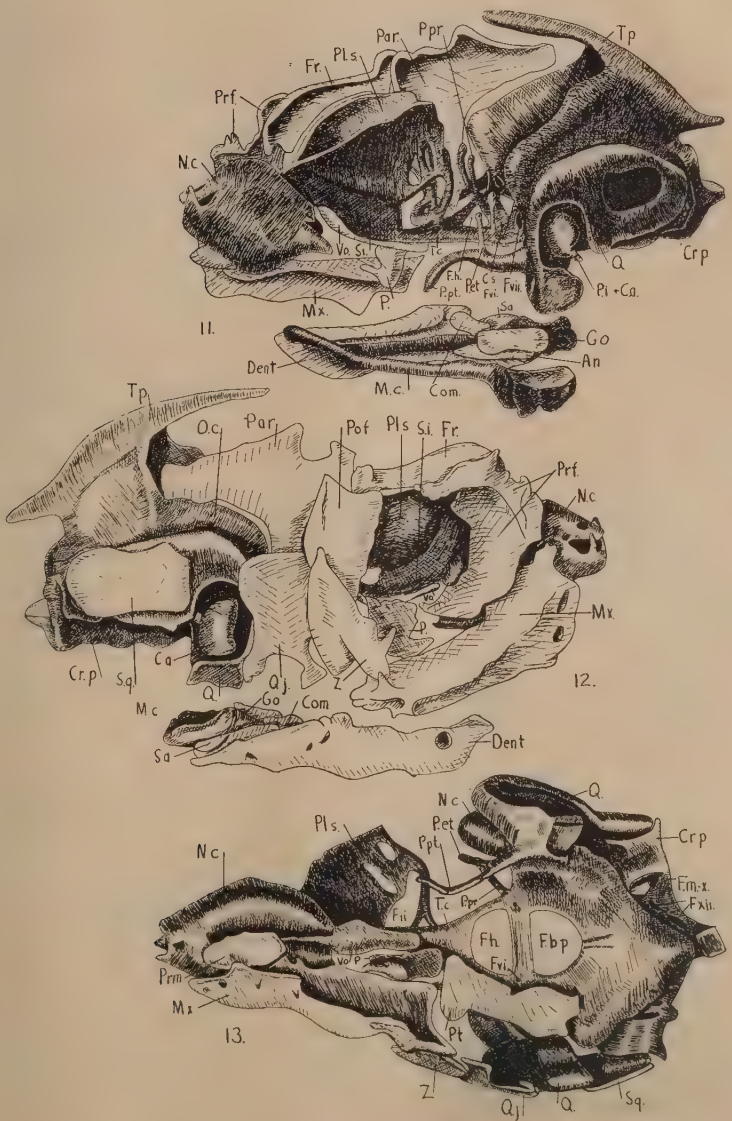
Group III. Parietal and frontal bones. These two complete the roof of the brain case.

Group IV. Prefrontal, postfrontal, zygomatic, parts of the palatine and of the maxillary bones. This well-defined group makes up the orbit.

Group V. Premaxillary bone. The premaxillary is the sole representative in turtles of the group of membrane bones that develop around the nasal capsule. In other reptiles the nasal and septomaxillary bones would fall here.

Group VI. Squamosal and quadratojugal bones. Both of these are closely related to the quadrate cartilage and bone.

Whether or no these groups have any significance outside reptiles the writer leaves to others more skilled in following the intricacies of skull development in other vertebrate classes. Of the value of the groups in understanding the reptile skull more will be said below.



Figs. 11 to 13 Wax model of the chondrocranium and membrane bones of a 19-mm. embryo of Chrysemys marginata, A.E.C. 103. × 11. 11, left side; 12, right side; 13, ventral side.

Cartilage bones

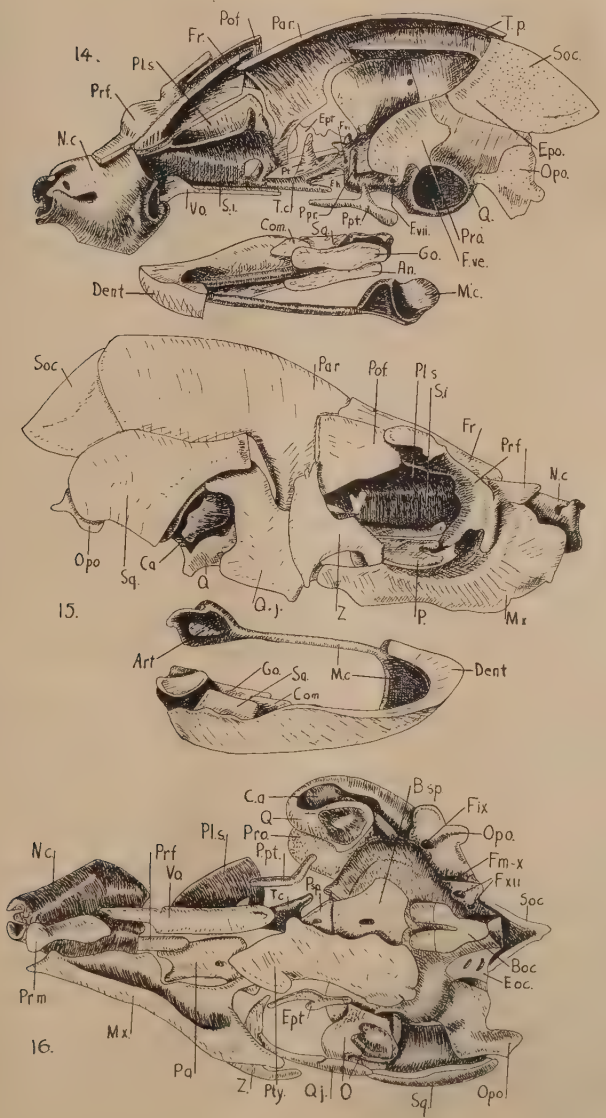
The bones which arise in cartilage are all found behind the hypophysis. They are shown in the oldest embryo modeled (28.5 mm., figs. 14, 15, 16).

The basisphenoid arises from two centers. The bone so formed fuses with the membrane-bone parasphenoid just anterior to it. The parasphenoid lies beneath the hypophysis; it closes off the hypophyseal fenestra of the chondrocranium. The lateral boundaries of the fenestra, i.e., the arms of the Y-shaped trabecula cranii (fig. 13), later ossify as cartilage bone and fuse with the parasphenoid beneath to form the shallow pocket in which the hypophysis rests. The adult basisphenoid is therefore formed of two cartilage-bone elements, the basisphenoid proper and the ossified parts of the trabecula cranii, and the membrane-bone parasphenoid.

The basioccipital also arises from two centers. The bone extends back into the occipital condyle. Anteriorly, the basioccipital annexes two delicate lamellae which lie beneath the cartilage floor and which are probably of membrane bone. The basioccipital in Chrysemys is always distinct from the exoccipitals. In Chrysemys the extreme lateral part of the cartilage floor, the crista substapedialis of Kunkel, seems to be replaced by a tiny ossicle that remains independent of the basioccipital.

The exoccipital or pleuroccipital bones probably ossify from several centers which could not be clearly distinguished. The bony matrix extends down into the side of the occipital condyle and up into the hypoglossal or exoccipital process. In Chrysemys the exoccipital does not fuse with the opisthotic.

The epiotic is continuous in the stage modeled with the supra-occipital, the ossification in the tectum. The two are usually described as distinct bones secondarily fused. No trace of secondary fusion appears in the 28.5-mm. embryo. The tectum, it should be remembered, is an extension of the otic capsules and has no connection with the occipital cartilage. In the 28.5-mm. embryo the supra-occipital likewise seems to be an extension of the two epiotics.



Figs. 14 to 16 Wax model of the chondrocranium, membrane bones, and cartilage bones of a 28.5-mm. embryo of Chrysemys marginata, A.E.C. 106. × 7. 14, left side; 15, right side; 16, ventral side.

The quadrate, exclusive of the pterygoid process, ossifies as the quadrate bone. Before ossification sets in, the cartilage beneath the squamosal degenerates, and the large hole left in the quadrate remains, although covered by the squamosal bone.

The pterygoid process breaks away from the quadrate cartilage (fig. 14) and ossifies into the tiny epipterygoid bone, which completes the lateral wall of the brain case.

Two more cartilage bones, not directly connected with the skull are found in the 28.5-mm. embryo. The articular bone appears beneath the quadratomeckelian joint in Meckel's cartilage (fig. 15). The operculum of the columella auris shows a trace of ossification. The extra-columella remains cartilaginous throughout life.

Degeneration of the chondrocranium

The chondrocranium behind the hypophysis is replaced by cartilage bones. The extra-columella is the only persisting cartilaginous structure in this region.

The pilae prooticae are degenerating in the 19-mm. and the 28-mm. embryo. They disappear without trace in the adult.

The rest of the chondrocranium, the trabecula cranii (except the parts incorporated into the basisphenoid), the interorbital septum and the planum supraseptale, which together made up the basket for the forebrain, are reduced to a set of membranes. The membranes retain the extent and shape of the cartilage, but are devoid of matrix and are made up of connective tissue. No ossicles develop in the interorbital septum.

The nasal capsule is also reduced to a connective-tissue membrane, which contains scattered groups of cartilage-like cells without matrix. The nasal septum is also largely membranous; it does contain, however, a delicate plate of hyaline cartilage.

NOTE ON FOSSIL SKULLS

The turtle skull, because of its simplicity and massiveness alike in the embryo and in the adult, lends itself very well to an embryological interpretation of the reptile skull. With its help, embryological criteria for many bones can be laid down, which may help to clear up homologies in fossil forms, and some of the more bizarre features of certain extinct species can be understood and explained.

Cartilage bones

Of the occipital- and otic-bone groups nothing need be said, for the criteria of these are obvious and homologies in this part of the skull rarely cause difficulty.

On the sphenoid group, however, the data in this paper can shed some light. 'Basisphenoid,' 'parasphenoid,' 'presphenoid,' 'orbitosphenoid,' 'alisphenoid,' and 'ethmoid' are terms applied by paleontologists to various bones that replace the crista sellaris and the prechordal chondrocranium. The terms, as used by many writers, are ill-defined and overlapping. The following criteria, some old, some new, may be of use.

The basisphenoid, in the narrower sense, is an ossification of the cartilage skull floor behind the hypophysis, in the crista sellaris. In many reptiles the cartilage floor and the basisphenoid bone have a lateral basipterygoid process on either side. The process is absent in turtles generally. The parasphenoid is a membrane bone which closes off the hypophyseal fenestra and (especially in primitive reptiles) extends forward beneath the trabecula cranii and the interorbital septum. The terms presphenoid and ethmoid should be applied to ossifications in the interorbital septum, the former restricted to bone formed in the posterior part of the septum; the latter, to bone laid down in that part of the septum adjacent to the nasal septum. The orbitosphenoid is an ossification of the planum supraseptale, above and behind the optic nerve and anterior to the oculomotor and trochlear nerves. The true

alisphenoid in reptiles is an ossification of the pila prootica, and hence lies between the oculomotor and trochlear nerves in front and the trigeminal nerve behind.

In paleontological literature the basisphenoid and parasphenoid are seldom identified incorrectly. The parasphenoid shows its fundamental nature very clearly in the cotylosaurs, for example. The presphenoid is typically developed in Lambiosaurus (Gilmore, '24) and in Edmontosaurus (Lambe, '20). In Corythosaurus Parks ('23, pl. IV) gives the name 'parasphenoid' to a large vertical blade which projects forward from the basisphenoid beneath the optic nerve. While the bone may have a parasphenoid edge, most of it is almost certainly replacing the trabecula cranii and the interorbital septum, and should be called a presphenoid. The term 'ethmoid' has been loosely applied to an ossification of the whole interorbital septum in Lacerta (Parker and Haswell) and in Diadectes (Case). In such cases 'presphenoid' is preferable; the term 'ethmoid' should be restricted to the rostral part of the interorbital septum, which may have some relation to the ethmoid of mammals, as Sollas and Sollas ('14) have pointed out in their monograph on Dicynodon.

An ossification of the part of the planum supraseptale behind the optic nerve—the metotic region—has been called the 'alisphenoid' by Parker ('79). Such a bone lies in the field of the orbitosphenoid. The true alisphenoid, in all modern forms in which its development can be followed, arises as an ossification of the pila prootica.

A more important source of confusion in the alisphenoid region is the question of the presence of an epipterygoid in place of an alisphenoid bone. A true alisphenoid ossification of the pila prootica is found in Tropidonotus (Parker, '78) and in the crocodile (Kesteven, '18). In Lacerta the cartilaginous pila prootica persists. In the turtle the pila disappears and the gap in the brain case is filled by an epipterygoid ossification of the epipterygoid and pterygoid processes of the quadrate (figs. 11, 14). In fossil forms one can sometimes determine which bone is present by noting

the slight differences in relations to nerves and bones which follow upon the differing developmental histories of the two bones.

The alisphenoid is an ossification of the pila prootica (fig. 11). The trochlear and oculomotor nerves lie anterior and the entire trigeminal nerve lies posterior to the pila. The alisphenoid takes over the same relations. The alisphenoid develops in the plane of the otic capsule, and hence is not apt to overlap the prootic bone and will not usually cover the foramen of the facial nerve.

The epipterygoid bone is an ossification of the epipterygoid and pterygoid processes of the quadrate cartilage. In the chondrocranium the maxillary and mandibular nerves cross the pterygoid process behind the epipterygoid process, but the ophthalmic turns forward along the inner side of the pterygoid process to reach the orbit. When the cartilage structures are replaced by the epipterygoid bone, the first part of the ophthalmic nerve is covered. Also, since the epipterygoid bone has a more lateral position, it tends to grow backward over the lateral surface of the prootic bone, and can cover the facial foramen, as it actually does in the turtle.

In Edmontosaurus (Lambe, '20) the alisphenoid clearly fits into the space in front of the prootic and the exits of the trigeminal and facial nerves are unobstructed. In Lambeosaurus (Gilmore, '24) the bone marked 'alisphenoid' extends back to the paroccipital (opisthotic). No facial foramen shows in the drawing. In the text Gilmore quotes Lambe as saying, "The ophthalmic branch of the trigeminal nerve is enclosed in bone in its forward course . . . ." In this instance the presence of an epipterygoid is very likely.

Membrane bones

The membrane bones of the higher vertebrate skull are descendants of ossifications found beneath the skin of the head and around the oral cavity of lower vertebrates, bones which at first had no close relation to the skull proper. In mammals this overskull has been intimately welded to the

cartilaginous bones. In reptiles one finds an interesting halfway stage in evolution, where the membrane bones retain considerable freedom in the adult and betray their origin very clearly in the embryo. From the embryonic grouping of the membrane bones some useful criteria for the adult bones can be established and some of the extraordinary developments of the reptile skull explained.

The group of bones laid down on the ventral aspect of the chondrocranium (group II) corresponds fairly well to the 'Zahnknochen' of Hertwig and Gegenbaur (Gaupp, '06)—a group thought to have arisen around the teeth. In reptiles their secondary relation to the brain case is more important. The separate bones are usually easily identified from their standard relation to one another and to the overlying brain case. The pterygoid may have a constant relation to the pterygoid process of the quadrate; in reptiles it generally encloses the palatine branch of the facial nerve, as Gaupp has pointed out.

The parietal and frontal bones (group III) complete the roof over the brain case. They can be identified by their standard relation to the cranial cavity. The two bones seem to be the most conservative in the reptile skull. Almost the extreme of variation can be found in the turtle and the snake. In the turtle the parietal sends a long process ventrally to fill in part of the vacant alisphenoid area. In the snake the frontal extends ventrally and does duty for absent orbitosphenoid—a defect correlated with the slight development of the orbitosphenoid (prechordal) region in snakes.

Group IV includes the prefrontal, postfrontal, zygomatic, and in part the maxillary bones, to which may be added the postorbital and lachrymal of other reptiles. All are subdermal ossifications that have at first a very loose connection with the skull. They are an early generation of sclerotic bones which are later incorporated into the skull to form a socket for the eye. No matter what changes take place in the skull, the members of this group retain their relation to each other and to the eye—a characteristic which serves to identify them in most diverse types of reptile skulls.

In the nasal group (V) the turtle skull has but one bone—the premaxillary. In other reptiles the septomaxillary and nasal bones would be included. All three bones develop as ossifications to cover the cartilaginous nasal capsule and have at first a very loose connection with the rest of the skull. The premaxillary lies on the ventrolateral (respiratory) surface, the nasal on the dorsal (olfactory) surface, and the septomaxillary lies beneath the ventromedial cartilage and the organ of Jacobson. In the turtle a process of the prefrontal takes the place of a nasal, and the absence of a septomaxillary is perhaps correlated with the doubtful presence of an organ of Jacobson.

The close relation of the three bones to the cartilaginous nasal capsule explains the formation by them of the extraordinary crests of the helmet-crested Hadrosauridae, e.g., Lambeosaurus and Hypacrosaurus (Gilmore, '20). The helmet in the crested dinosaurs contains an hypertrophied olfactory apparatus. The premaxillary and nasal bones have only developed with the organ around which they are laid down in the first place.

The nasal capsule and its related bones are semi-independent of the rest of the embryo skull, and can hypertrophy without affecting the rest of the skull. It is unlikely that the frontal bone, for example, would follow the nasal capsule and participate in the formation of such a crest, as Parks ('22) thinks the bone does in Parasaurolophus.

The last group of membrane bones (VI), the squamosal and quadratojugal, are definitely associated with the quadrate cartilage and bone. The squamosal appears on the posterior and dorsal aspects of the quadrate cartilage and the quadratojugal develops along its anterior margin. In later stages (fig. 15) the squamosal overlaps the quadratojugal along the dorsal margin of the quadrate. These are the embryonic criteria for the bones that Thyng ('06) has established for vertebrates generally.

Temporal arches and vacuities

The grouping of the membrane bones in turtle embryos suggests an explanation for the origin of the temporal arches and vacuities. In nearly every case the temporal arches can be thought of as an arrangement whereby the quadrate-squamosal-quadratojugal group is fixed to the cranium and its roof bones on one hand and to the orbital group of bones on the other. The object would seem to be the shoring of the quadrate-mandibular joint. Bony tissue characteristically increases along lines of stress and strain, and disappears where these are absent. The arches seem to be placed on such lines of stress and strain which would appear when the mandibular joint is functioning; the vacuities mark elements of the stegocephalian membrane-bone overskull which are not needed in the more compact reptile skull. It is true the turtle is aberrant in this matter; its bony arches do not easily lend themselves to comparison with those of other reptiles. The turtle is an exception, however, which helps prove the rule. The turtle quadrate is unusually broad and flat, and is well fixed to the otic bones. Hence the temporal arches are less needed and are more variable.

Such is, of course, not the whole story of the temporal arches. The writer is in no sense of the word an expert on fossil reptiles. It does seem to him, a student of the reptile embryo, that the paleontologist lays too much stress on the vacuities and the loss of such loosely articulated bones as the intertemporal. Both of these are secondary to the arches and the reinforcement of the quadrate-mandibular joint in a type of skull that is changing from the loosely articulated amphibian sort to the compact unified skull of higher vertebrates.

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THE TRANSFORMATION OF LYMPHOCYTES INTO ERYTHROBLASTS IN A LYMPH NODE OF A RABBIT

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THREE PLATES (ELEVEN FIGURES)

The material of this investigation was obtained under the following circumstances: A full-grown albino rabbit was secured from the animal pen of the Department of Pharmacology for the purpose of studying fresh areolar connective tissue in the course in histology. After the laboratory period the submaxillary salivary glands and some femoral marrow were removed and fixed in Helly's fluid. These tissues were subsequently given to the laboratory technician with instructions to prepare sections stained with hematoxylin and eosin for the student loan collection of slides. When the sections were submitted for examination I noticed a peculiarly altered lymph node attached to one of the salivary glands. More careful study with higher magnification revealed at once that this particular lymph node was undergoing extensive erythroblastic metaplasia. Subsequently, other sections were stained with the Giemsa mixture and with the iron-hematoxylin eosin combination.

The present report concerns itself primarily with the unequivocal evidence relating to the direct transformation of lymphocytes into erythrocytes. Moreover, this lymph node reveals suggestive evidence in regard to the specific differential factor underlying red-cell development and the manner in which extravascularly differentiating erythrocytes may subsequently enter the medullary vascular channels. It is, of

course, unfortunate that there are no data on record concerning the previous treatment or experience of this rabbit, that a careful postmortem examination of the viscera was not made, and that other tissues, especially other lymph nodes and the spleen, were not removed for histological examination. However, the rabbit appeared well nourished and in good health, and there is no reason to suspect that he had been ill or used for experimental purposes. Luckily, sections of bone-marrow and of blood vessels in the submaxillary glands are available for comparison with the erythrocytopoietically active lymph node.

In a number of previous joint communications I have urged that the lymphocyte represents a relatively undifferentiated cell endowed with multiple developmental capacities, one of which is the ability to differentiate under favorable circumstances directly into an erythrocyte. I have suggested, further, as a reasonable working hypothesis that the lymphocytes normally perform a large portion of their total function by differentiating into erythrocytes after they are filtered out of the blood stream in the red bone-marrow. If it can be demonstrated, as in the case of the lymph node here to be described, that lymphocytes do under certain conditions differentiate into erythrocytes, then the hypothesis just mentioned receives weighty additional support, and the question of the normal fate of the lymphocyte may receive its proper and complete answer.

DESCRIPTION

This lymph node has an oval shape, measuring approximately 5 mm. in length and 2 mm. in thickness (fig. 1). The available sections show no distinct hilum. The largest arterial vessel lies along the inner border of the altered cortex. A smaller vein leaves the node from the opposite surface. Apparently, the blood vessels enter and leave the node over a wide area. The node is covered with a thin capsule of loose areolar connective tissue continuous with the stroma of the submaxillary salivary gland. Longitudinal

sections present vague evidence of a division into a cortical and a medullary portion.

The cortical portion comprises a thin oval discoid mass of more compact 'lymphoid' tissue over one surface of the node. This cortical disc measures approximately 2 mm. in its longest diameter, and has an average depth of approximately one-third of a millimeter (fig. 1). Peripherally, it presents a sharp contour; internally, its boundary is very irregular and more or less vague in certain areas (fig. 2). No definite peripheral sinus is discernible. The disappearance of this sinus is presumably the result of an intense proliferative activity of the cortical lymphocytes, the new-formed lymphocytes having become crowded into the original peripheral sinus. Whether this lymphocyte increase preceded, followed, or accompanied the atrophy of the afferent lymphatics remains uncertain. Theoretical considerations outlined below would seem to favor the primary atrophy of the supplying lymphatics.

The lymphocytes of the cortex are predominantly of the small variety. Only relatively few large lymphocytes and erythroblasts occur within this area. The differentiating erythrocytes are in general in the close vicinity of the blood vessels. The latter are of relatively large caliber and comprise both arteries and veins. The red blood corpuscles in some of the cortical vessels and many of the medullary vessels stain only very faintly except with iron-hematoxylin, appearing mostly as rings resembling 'blood shadows' of laked blood. The laking of the blood in many of the vessels presumably indicates stagnation. The intranodal blood vessels contain very few of the specific cells of this altered node, namely, differentiating erythroblasts.

The differentiating lymphocytes are more compactly aggregated immediately about the blood vessels. The latter are largely of the thin-walled sinusoidal type. Large portions of the cortex stain very lightly (fig. 1). This differential staining reaction follows from a peculiar character of the lymphocytes. The small lymphocytes of these lighter areas have

vesicular nuclei, and their cytoplasm also stains only faintly. The appearance is somewhat that of the peripheral portion of the cortical nodules of very active lymph nodes. But there is nothing in this cortical area to suggest definite lymph nodules or germinal centers. The greater bulk of the anomalous cortex apparently corresponds in regard to its predominating cells to the peripheral portions of the cortical nodules of normal lymph nodes. However, many of these cells are clearly in process of degeneration, as indicated by a considerable amount of karyorrhexis or a partial resorption of the nucleus. Some of the cells apparently represent abortive erythrocytes, incapable of elaborating hemoglobin.

The most plausible interpretation of the modified cortex would seem to be in terms of an initial intense proliferation in original cortical nodules, effecting secondary fusions and an invasion of the peripheral sinus, followed by a gradual drainage and erythroblastic differentiation of lymphocytes along the medullary border. However, mitotic figures are entirely lacking, and evidence even of amitotic proliferation is meager. Reconciliation of this contradictory fact with the interpretation suggested on other grounds may be effected on the assumption that the period of lymphocyte multiplication considerably antedated the time when this node was removed. This assumption receives support from the fact of widespread regressive changes in the medulla, as will be described below. The further apparently contradictory fact of an abnormally thin cortex may be explained as the result of a central drainage of lymphocytes following the closure of the afferent lymphatics and preceding the later atrophy of the efferent lymphatics.

The medulla consists of wide irregular areas of very diffuse lymphoid tissue and variable narrow cords of compact lymphoid tissue (figs. 2 and 3). The former most probably represent original medullary sinuses, secondarily enlarged by gradual reduction of the medullary cords, both peripheral and central. The reticular stroma of the altered sinuses is wide-meshed. The reticular cells have for the most part an

unhealthy atrophic or hydropic vacuolated appearance (figs. 7 and 8). In certain small areas near the ends of the node the intercellular spaces appear filled with a mucoid substance.

The parenchyma of the medullary sinuses includes predominantly scattered erythroblasts at various stages of differentiation (fig. 6), an occasional apparently unaltered lymphocyte, numerous cells with budding (fragmenting) nucleus (fig. 6, *f*), and certain larger mononuclear and binucleated cells with vacuolated cytoplasm, probably representing free reticular cells, and of the nature of plasma cells (figs. 6, 7, and 8). Obviously, the conditions here are those largely of regression and beginning degeneration. There is, however, no evidence of phagocytosis, nor are erythroplastids to be seen in these areas. The interpretation that the histologic conditions suggest is disconnection of afferent lymphatics, followed by almost complete drainage of the sinuses of their original lymphocyte content, and subsequent atrophy of the efferent lymphatics. Certain of the persistent lymphocytes of the isolated medullary sinuses differentiate into erythroblasts (fig. 6, *c, d,* and *e*). The node contains no fat-cells.

The central point of interest in this anomalous lymph node concerns the histology of the medullary cords (figs. 4, 9, 10, and 11). Many of these contain an axial blood vessel (figs. 4 and 9), others lack all trace of a vessel (fig. 11), and a few are in a condition of transition, showing a phase of the process of obliteration of the central vessel through disruption of the endothelium and cellular invasion of the original lumen (fig. 10).

The predominating cell in most of these cords is the young erythroblast, the normoblast (fig. 5, *b*), with a relatively small granular spheroidal nucleus, and a considerable envelope of slightly acidophilic cytoplasm. With the hematoxylin-eosin staining technic the cytoplasm of these cells colors a variable, but generally deep shade of red, with a variable bluish tinge. In terms of the amount of hemoglobin, as indicated progressively by the loss of bluish tinge and a deepening pink color

of the cytoplasm, the normoblast has apparently an approximately normal content. Only relatively few cells, however, pass beyond this stage of differentiation as regards hemoglobin elaboration, though nuclear changes proceed in many cells to late erythroblast conditions (fig. 5, *c* and *d*). Occasional erythroblasts contrast sharply with the surrounding cells, by reason of their brilliant red color, indicative of a large amount of hemoglobin (fig. 11, cell *xx*). Intermediate stages between the latter and the slightly basophilic younger normoblasts are relatively numerous (figs. 10 and 11, cells *x*). In the Giemsa-stained preparations the majority of the cordal cells stain various shades of blue; only the older erythroblasts show a pinkish cytoplasm. From among the cells of almost any cord a closely graded series of transition stages may be arranged leading from a typical medium-sized lymphocyte (fig. 5, *a*) through typical normoblast stages (fig. 5, *b*) to older erythroblasts in which the nucleus is undergoing fragmentation and resorption (fig. 5, *d*).

A type of cell which was at first confusing is one in which the nucleus has sprouted small spheroidal buds which remain connected with the central chromatic mass by delicate threads, giving to the structure as a whole a radiate appearance (fig. 6, *f*). These cells, which occur especially numerously among the more peripheral erythroblasts of some of the medullary cords, suggest in the more irregular conditions (fig. 5, *e*) either polymorphonuclear leucocytes or cells in mitosis. Their cytoplasm, however, lacks granules; their resemblance to amphophil polymorphonuclear granulocytes is only superficial. Moreover, the complete absence of such cells with chromatic masses arranged in the form of diasters (anaphase of mitosis) makes it quite clear that the condition is not indicative of indirect division. The slight degree of proliferative capacity persisting at the time this node was removed was of the direct type, as suggested by occasional binucleated erythroblasts (fig. 5, *f*).

Nor are these cells properly interpreted as phases of the enucleation process by which the erythroblast becomes an

erythroplastid. This process is practically exclusively one of intracellular resorption (fig. 5, *c*, and cells below *e*), sometimes preceded by a previous nuclear fission (fig. 5, *d*). The cells in question are unmistakably in process of degeneration, accompanied by karyorrhexis. No unequivocal instances either of erythroplastids or granulocytes occur anywhere within this lymph node. Occasional cells like that of figure 7 with a few small spheroidal granules are puzzling, but most likely also represent a phase of degeneration. In Giemsa-stained preparations these granules have a red color and the cells have the appearance of eosinophilic granulocytes. The conditions in this node are such as to support erythrocyte differentiation practically exclusively; and this only inadequately, as indicated by the slowness and frequently abortive issue of the process, in the case of only a very few cells leading to a normal hemoglobin content and complete enucleation.

The medullary cords are without exception enveloped by what appears to be a typical endothelial membrane (figs. 4, 9, 10, and 11). This is obviously derived from the reticulum, possibly largely through the operation of the mechanical factor of pressure. The occurrence of transition stages (fig. 10) between cords with an axial blood vessel (figs. 4 and 9) and such as lack any trace of blood vessel (fig. 11) indicates either that the cords were originally solid and became secondarily invaded by a blood vessel or that the original blood vessel of the cords suffered obliteration. In either event, as shown by the transition stages (fig. 10), the endothelium is locally fenestrated to permit the entrance to its lumen of the peripherally differentiating lymphocytes. The evidence favors more the interpretation that the endothelium of the central vessel suffers disruption and that its lumen becomes obliterated through erythroblast invasion. In this manner the new-formed reticulum-endothelium becomes continuous proximally with the proper endothelium of the cordal blood vessel, and the cells of this distal 'sinusoid' are placed in direct communication with the terminal blood channels. Even in figure 9 there is apparently a small gap in the endothelium

at the right, through which several erythroblasts have entered the lumen. The evidence from normal histology, as regards the blood supply of lymph nodes, also favors the interpretation that these medullary cords were originally vascularized columns of lymphocytes. The lymphocytes evidently differentiated slowly into erythroblasts under the stimulus of some specific transudation product from the plasma of the included blood vessel.

In the Giemsa-stained sections only some of the more peripheral medullary blood vessels contain deep-red-stained corpuscles; elsewhere the corpuscles appear laked of their hemoglobin. Obviously, the blood circulated very poorly through this node. This condition of essential blood stasis operated to produce a local accumulation of carbon dioxide. In the resulting relatively high concentration of carbon dioxide most probably inheres the specific stimulus for hemoglobin elaboration.

DISCUSSION

The only plausible interpretation of this structure, as the above description suggests, is in terms of an altered lymph node. The modification obviously consists primarily in an atrophy of the afferent lymphatics and a subsequent drainage of the lymph sinuses, involving the deeper portions of the cortex and the more peripheral portions of the medullary cords, followed by atrophy of the efferent lymphatics. The blood supply has largely remained intact in spite of certain regressive changes consequent to the drainage and isolation of the lymph sinuses. It apparently spread somewhat originally, stimulating thus proliferation of lymphocytes, especially in the cortex, which caused obliteration of the peripheral lymph sinus. Subsequently, certain vessels suffered partial or complete isolation, as indicated by the stagnation of their blood current, presenting thus the specific conditions determining an erythroblastic metaplasia of the enveloping lymphocytes.

Three possibilities present themselves in explanation of the alteration of this lymph node: First, it might conceivably represent a transition stage between a lymph node and a hemal node; secondly, it might represent a compensatory reaction of potentially hemopoietic tissue to a defective or inadequate bone-marrow; thirdly, it might represent a regressive phase of a transient lymph node. The last possibility involves the further alternative of complete degeneration and ultimate removal or partial degeneration to be followed by complete regeneration.

The altered vascular conditions in this lymph node are exactly those which von Schumacher ('12) believes lead to the transformation of an ordinary lymph node into a hemal node, namely, atrophy of the afferent lymphatics. Under such circumstances the blood vessels are believed to effect connections with the original lymph sinuses and to cause these to become filled with blood. In the lymph node here under discussion such result did not follow. The blood is limited to the blood vessels. Moreover, the differentiating erythroblasts are passing into the blood vessels, not into the almost empty lymph sinuses. The latter contain only scattered erythroblasts, differentiation products of lymphocytes trapped within the sinuses after atrophy of the efferent lymphatics. Meyer ('17) denies that transitions occur between lymph nodes and hemal nodes. He defines a hemal node as a mass of lymphoid tissue intercalated from the beginning in the venous blood system and lacking both primitively and definitely any lymphatics. Conceivably, this node might yet have developed into a hemal node, but regressive changes within the medullary sinuses appear to me to have proceeded too far to render such a developmental outcome at all likely. A single exception does, of course, not disprove the hypothesis of hemal node derivation from lymph node, but this particular node, in which the chief theoretical conditions are met as regards the vascular and lymphatic supply, offers no support. However, certain hemal nodes in my collection give rather clear evidence of lymph-node derivation. Also, in these altered nodes, at-

tached to sections of the human parotid, there occur regions of lymphocyte transformation into erythrocytes. Since a complete description of these hemal nodes involves very numerous peculiar giant cells here present, the entire matter will be reserved for a separate study.

Consideration of the second explanatory possibility of this node involves a description of the bone-marrow. The specimen of marrow from this individual available for comparison with the lymph node is from the femur. It differs from the same marrow of a number of control specimens in its complete absence of fat. Moreover, it is predominantly granulocytopoietic. The granulocytes are almost exclusively of the amphophil variety. The complete absence of fat might signify compensatory reaction to a severe anemia. The predominance of amphophilic granulopoietic activity may indicate extensive bacterial infection. The presumed infection would most probably entail anemia. These suggestions may explain the peculiar conditions of this marrow. Assuming that all of the lymph nodes of this individual, and possibly also the spleen, were in functional conditions similar to that of the altered submaxillary lymph node, as indicated by its histology, then the conclusion would logically follow that this modification of the lymphoid tissue was compensatory to the primary condition of an abnormal bone-marrow. In other words, given the conditions imposing a predominantly granulopoietic function upon the bone-marrow, the regulatory capacity of the body would force an erythrocytopoietic activity upon the uninvolved potentially hemopoietic tissue, namely, the spleen and lymph nodes. Absence of the pertinent information regarding other portions of the lymph-adenoid system greatly invalidates any explanations attempted from this approach. If erythrocytopoietic compensation to a defective myeloid tissue were the correct interpretation of the condition of this lymph node, then one would expect the node in question to be packed with erythropoietic tissue rather than with merely scattered islands of erythroblasts. However, this objection assumes that all of the lymph nodes are

in essentially the same condition as the specimen removed. On the contrary, other nodes, and perhaps the spleen, may be much more active in red-cell production; this submaxillary node may have arrived at a stage nearing exhaustion. Moreover, the marrow may just be entering upon a more nearly normal hemopoietic function, as suggested by the presence of some erythroblasts among the granulocytes.

Ignoring for the moment the striking, and assuredly significant, division of the two chief aspects of hemopoiesis between this lymph node and the specimen of femoral marrow—the former being exclusively erythrocytopoietic, the latter predominantly granulocytopoietic—we may next consider the third possibility above listed regarding the explanation of this submaxillary lymph node. This possibility interprets this structure as a regressive phase of a transient lymph node. If we accept Gulland's ('94) view of lymph nodes as unstable and evanescent structures, which appear and disappear as needed, then this node could take a very definite place in the series of alleged stages leading to complete or partial disappearance. Whatever the actual status of this view may be, this particular node, judged by the apparent degree of degenerative changes within the medullary sinuses, and even portions of the cortex, represents a relatively late phase in the regression of a transient lymph node. It is difficult for me to conceive of the restoration of this structure to a typical lymph node, and it seems more in accord with the histological data to consider it doomed to final resorption.

However this may be, the central point of our present interest is not materially affected. This point concerns the transformation of lymphocytes into erythrocytes under the peculiar structural, chiefly vascular, conditions of this node. This material is of prime value and importance in that it demonstrates erythrocytogenic capacity of lymphocytes, given favorable conditions, and gives very suggestive evidence concerning the specific differential factors mediating hemoglobin elaboration.

In a joint communication with Doctor Marshall ('25), I reported a condition of myeloid metaplasia of lymph nodes in a case of atypical anemia (leukemia?) in which lymphocytes differentiate directly into erythrocytes within the nodes. These nodes contain great numbers of nucleated red cells and abundant examples of closely graded series of transition stages between typical small and medium-sized lymphocytes and typical normoblasts. The conclusion of in situ differentiation of erythrocytes from the lymph-node lymphocytes was the more definite by reason of the clearly degenerate condition of the marrow, the functional incapacitation of the spleen by radium irradiation, and the almost complete absence of nucleated red cells from the circulation. Similarly, in the case of certain fishes and amphibia, Jordan and Speidel ('23, '24, '25) describe direct transformation of lymphocytes of the spleen into erythrocytes. Laguesse ('90) also derives the erythrocytes of fishes from splenic lymphocytes. Badertscher ('15) describes a similar transformation of large and medium-sized lymphocytes into erythrocytes in pig fetuses from 55 mm. to full term.

The studies of Alder and Huber ('23) present strong confirmation of our claim that the lymphocytes of amphibians function as hemocytoblasts. They describe this same cell in the spleen, liver, and bone-marrow of various amphibians and reptiles in its process of differentiation into both erythrocytes and granular leucocytes. However, they do not interpret this cell as a true lymphocyte. They name it 'hemocytoblast,' and give it as their opinion that genuine lymphocytes do not occur in forms below birds. But this is simply a matter of terminology, and their descriptions and illustrations only seem to me further to support the view of an erythrocytogenic capacity of the lymphocyte.

Moreover, Chlopin and Chlopin ('25) have very recently shown in tissue cultures of the spleen of axolotl that even under these conditions the splenic lymphocytes differentiate into both erythrocytes and granular leucocytes. To be sure, as regards the erythrocyte, they describe its derivation

directly from a 'hemohistioblast,' a slightly differentiated reticular cell. But at the same time they derive also the small and large lymphocytes from a reticular source. Judging from their illustrations, the larger lymphocytes and the later hemohistioblasts (early erythroblasts) are essentially identical. In the case of the special and eosinophilic granulocytes they derive these cells from both lymphocytes and reticular sources, and in my opinion their data should have led them to the same conclusion regarding the erythrocytes.

The atypical, possibly pathologic, lymph node above described gives even more unequivocal evidence that under certain conditions lymphocytes differentiate in situ into erythrocytes. The close structural similarity, amounting in certain cases to an apparent identity, as in the case of Amphibia, between the ancestral blood cell of the marrow and the lymphocytes of the spleen, and the similarity of structural conditions, as regards particularly the character of the blood supply, in the various hemopoietic loci (yolk sac, liver, spleen, mesonephros, and red bone-marrow) suggest that lymphocytes of mammals are normally in large part filtered out of the blood stream within the bone-marrow to function here as the ancestors of both erythrocytes and leucocytes. This suggestion receives indirect support from the fact that as yet no other hypothesis regarding the fate of the lymphocyte has been able to meet the objection of numerical disparity between the enormous numbers of lymphocytes known to leave the blood stream daily and the relatively small number whose disappearance can be accounted for on the basis of actual observations. This matter is more fully discussed in a previous paper (Jordan and Speidel, '24).

Much of the recent work on leukemias also leads to the conclusion that, under the conditions prevailing in this type of disease, lymphocytes of lymph nodes differentiate into 'myeloid cells.' Under the latter term is usually understood to be included only granulocytes at various stages of development. Though the term 'myeloid' should properly include

also erythroblasts, statements that these same lymphocytes differentiate also into red cells are generally given only in uncertain and qualified terms. Citron ('15), in his discussion of a case of 'micromyeloblastic leukemia,' states his belief that in some cases a direct autocellular change of lymphatic follicular lymphocytes into myeloid cells may take place in both spleen and lymph nodes. Fineman ('22), in his case of microlymphoidocytic leukemia, notes the abundance of transition forms between lymphocytes and hemoblasts ('atypical cells') both in the peripheral blood and in the lymph nodes, but is unable to determine whether the lymphocyte is the mother cell of the atypical cell or vice versa (p. 5). Logefeil ('24) finds evidence in a case of 'mixed leukemia' of a "direct transition from lymphocytes to myelocytes, without going through the stage of the stem cell" (hemoblast). He notes also evidence of local development of myelocytes from lymphocytes in the areas of leukemic infiltration in the pancreas, kidneys, and lungs. Furthermore, both in the lymph nodes and the spleen he found immature lymphocytes and myelocytes diffusely arranged without evidence of segregation. He states also that many of the eosinophils, both immature and adult, had nuclei identical with adjacent lymphocytes. He quotes Turk ('08) as having reported many nucleated reds in the lymph nodes in a case of mixed leukemia. But no definite statement appears as to whether these reds are believed to be the result of congestion, infiltration, or local in situ differentiation. Finally, Downey ('24) states that under pathological and experimental conditions which cause myeloid metaplasia the derivation of myeloid cells from lymphocytes without the intervention of the myeloblast may be an extensive process. He admits further that the blood in lymphatic leukemia may show all transition forms from the 'myeloblast' (hemoblast) to the ordinary lymphocyte, without, however, committing himself as to which cell is the progenitor.

The claim that lymphocytes function in part as erythrocyte ancestors within the bone-marrow does not exclude other sources of origin. No doubt, endothelium functions in part

in the same manner. That young endothelium has a potential erythrocytogenic capacity is demonstrated in the case of the so-called aortic cell-clusters of mammals ('16, '17). Similar endothelial blood islands were seen by Sabin ('17) in the aorta of the living chick embryo of the second day. Furthermore, Doan ('23) has described an intravascular endothelial origin of erythrocytes in the bone-marrow of the adult pigeon; and Cunningham and Doan ('23) have arrived at the same conclusion regarding the bone-marrow of the adult rabbit. The double facultative origin of erythrocytes from lymphocytes and endothelium is readily explicable on the basis of the close genetic relationship between the two types of cells and their common only slight degree of differentiation from the ancestral mesenchyme. The pluripotential developmental capacity of the mesenchyme includes direct blood-cell production, lymphocyte production, and endothelium production. The interchangeable functional capacity of these tissues accordingly inheres in the maintenance of a considerable degree of original polyvalency. Moreover, the development of a membrane about the medullary cords above described, indistinguishable in these sections from endothelium, demonstrates again the close genetic relationship between reticulum cells and endothelium, and emphasizes the need for extreme caution in interpreting endothelium-lined spaces of marrow as originally continuous with the blood capillaries.

Another matter of primary interest in this peculiarly modified lymph node relates to the specific conditions of hemopoiesis and to the essential identity of the process of erythrocytopoiesis here and that described by Latta ('21) in the iliac tonsil (Peyer's patches) of young rabbits. This important work of Latta has not yet received the attention that its results would seem to merit. Latta shows that the fate of the lymphocytes in the primary iliac patches is dependent upon the blood supply and the closeness of their relation to the blood vessels. If the relation is remote, development proceeds in a random fashion and diffuse lymphatic tissue results. If the blood supply of any focus is particularly

good, the lymphocytes proliferate rapidly and aggregate about the blood vessels, forming dense lymphatic tissue. The further differentiation of the lymphocytes is dependent upon: 1) the closeness of association with the blood vessels, 2) the slowness of the current in the blood stream, and, 3) the thinness of the vascular walls. If these three conditions are very good, erythrocytopoiesis results; if poor, granulocytopoiesis occurs.

In the altered submaxillary lymph node the three conditions specified by Latta as requisite for erythrocytopoiesis are all adequately met. The lymphocytes have obviously undergone extensive proliferation about the blood vessels of the cortex; those of the medullary cords especially are closely associated with the axial blood vessels; the blood current is relatively stagnant, as indicated by the pale character of the erythroplastids in certain of the channels, and the vessel walls are uniformly thin. The primary factor involved in stimulating erythrocytopoiesis under these conditions, as pointed out by Latta, is probably a transudation product of the blood plasma. Elsewhere we have given the evidence in support of the conclusion that the specific erythrocytogenic factor or hormone resides primarily in a relatively high concentration of carbon dioxide in the regions of hemopoietic tissues (Jordan and Speidel, '24; Jordan, '24). Dallwig, Kolls, and Loevenhart ('15) have demonstrated that oxygen lack and carbon-dioxide excess in the respired air are both efficient stimuli for increase of erythrocyte production in red bone-marrow, but they regard the decrease of oxygen tension as the primary and more effective stimulus. However, a fall in oxygen tension of the respired air would secondarily effect an accumulation of carbon dioxide in the tissues, and an increase of carbon dioxide in the respired air, unless excessive, would be ineffective as a hemopoietic stimulus until after saturation of the buffer substances of the circulating blood. The specific structural features of this lymph node, as pertains especially to the stagnant blood current and the regressive degenerate condition of the medullary sinuses, give further support to the

hypothesis which locates the specific erythrocytogenic factor in the relatively increased concentration of carbon dioxide.

SUMMARY

1. An altered submaxillary lymph node of an adult albino rabbit is described. The principal modifications of the node consist in the atrophy of the lymphatics, the almost complete drainage of the medullary sinuses, and the erythroblastic differentiation of the lymphocytes of the medullary cords and certain perivascular areas of the thin cortex.

2. The predominating cell type of the medullary cords is a typical normoblast. Occasional older erythroblasts with considerable amounts of hemoglobin also occur. The various cells include closely graded series of transition stages between typical small and medium-sized lymphocytes and enucleating erythroblasts.

3. The majority of the cords contain a thin-walled axial blood vessel. A few are in transition stages to avascular conditions, and show a disruption of the endothelium and an invasion of the lumen by differentiating erythroblasts. The cords are enveloped by an endothelioid layer derived from the recticular stroma. This layer becomes continuous proximally with the endothelium of the intact portion of the involved blood vessel. The phenomenon suggests the mode by which extravascularly differentiating erythrocytes may enter the vascular capillary terminals of hemopoietic tissues.

4. The specific structural and functional conditions of this node under which the lymphocytes are stimulated to differentiate into erythrocytes involve the close association between lymphocytes and thin-walled blood vessels with relatively stagnant blood flow. These conditions represent the optimum for the diffusion of transudation products of the blood plasma among the surrounding lymphocytes. These vascular conditions also are such as provide for a relatively high concentration of carbon dioxide.

5. The bone-marrow also of this rabbit is peculiar in that it lacks fat entirely, and the hemopoietic activity is pre-

dominantly of the amphophil granulocytic type. The exclusively erythrocytopoietic activity of the lymph node and the predominantly granulocytic activity of the marrow may have causal relationship.

6. The data derived from the microscopic study of this anomalous, perhaps pathologic, lymph node support the hypothesis of an erythrocytogenic capacity of the lymphocyte under certain conditions, comparable to those which normally obtain in red bone-marrow and the fetal spleen. They support also the hypothesis which locates the specific erythrocytogenic stimulus in a relatively increased carbon-dioxide concentration within hemopoietic tissues.

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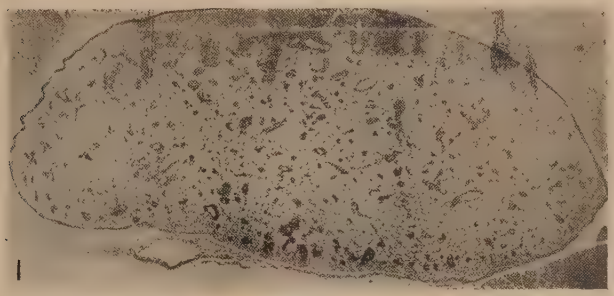
PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section, approximately medial, of altered submaxillary lymph node of rabbit, showing general shape and gross internal arrangement. The capsule is thin and loose. The dark irregular areas within the node represent remnants of cortex (above) and medullary cords. The lighter spaces seen near the center of these dark compact areas are blood vessels. Zenker-formol fixation; hematoxylin and eosin stain.

2 More highly magnified area from figure 1, showing cortex (above) and portion of the medulla. Note the blood vessel in many of the compact areas and the relatively wide and very loose intercordal areas of the medulla.

3 Highly magnified area from medullary portion shown in figure 2. The cord shown below, at the left, contains a relatively large, thin-walled blood vessel. The cord at the right, near the midpoint, is solid; its original blood vessel has become obliterated through the proliferation and subsequent invasion of the enveloping cells, the differentiating erythroblasts.



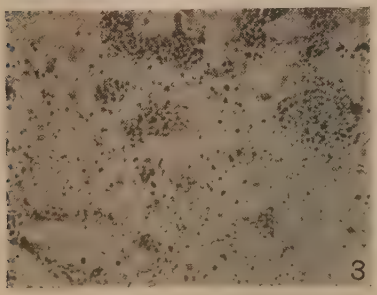


PLATE 2

EXPLANATION OF FIGURES

4 Transverse section of a small medullary cord, showing a central capillary blood vessel and a peripheral endothelioid reticulum membrane. The cells of the cord include erythroblasts at various stages of differentiation. × 1200.

5 Various cells from a medullary cord. *a*, lymphocyte (lymphoid hemoblast); *b*, normoblasts; *c*, erythroblasts; *d*, early stage in nuclear fragmentation and resorption of erythroblast; the cytoplasm contains relatively much hemoglobin, apparently largely in the form of globules; *e*, abortive erythroblasts in karyorrhexis. Surrounding *e* are six relatively small erythroblasts (microcytes) at various stages of differentiation.

6 Various types of cells from the intercordal areas of the medulla. *a*, large, binucleated, modified reticular cell; *b*, free reticular cell (monocyte?); *c*, normoblast; *d*, young erythroblast; *e*, two older erythroblasts; *f*, abortive erythroblast, showing karyorrhexis.

7 Small typical area of less extensively modified intercordal medullary area, showing three reticular cells and two erythroblasts.

8 Typical intercordal medullary area. The modified reticular cells are characterized by irregular shape and pale-staining vacuolated cytoplasm.

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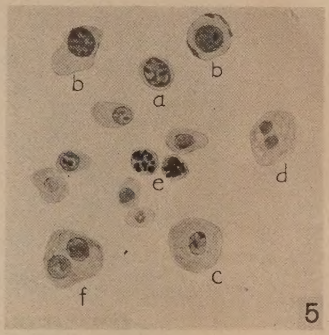
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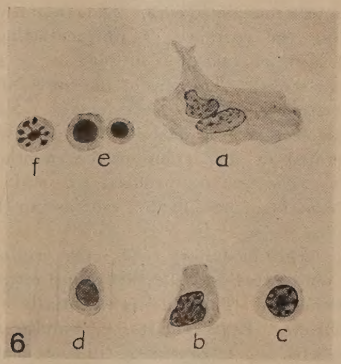
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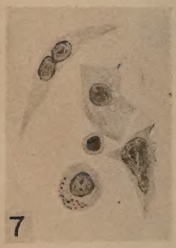
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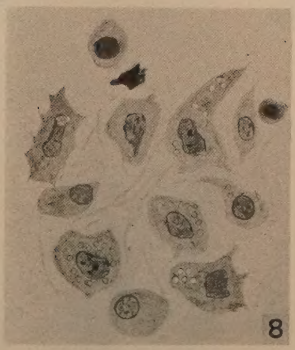
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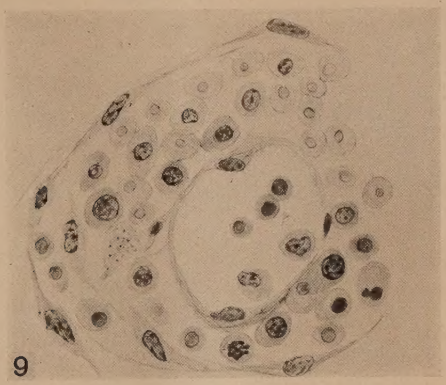
PLATE 3

EXPLANATION OF FIGURES

9 Transverse section of a medullary cord. The central blood vessel is defective at the right, permitting the entrance of differentiating erythroblasts. The periphery of the cord is fairly sharply outlined by a secondary endothelium derived from the reticular stroma. The cells of the cords include various transition stages in the differentiation process of erythrocytes. × 1200.

10 Similar transverse section of a medullary cord in which the central blood vessel is largely obliterated by disruption of the endothelium and invasion of lumen by erythroblasts. The two erythroblasts, *x*, stain a deeper pink color, indicative of a greater content of hemoglobin, contrasting thus with the bluish-red color of most of the cells.

11 Transection of a larger medullary cord. The original central blood vessel has become completely obliterated and the enveloping reticular tissue has become differentiated into endothelium. This cord is essentially a wide sinus filled with differentiating erythroblasts. Erythroblasts, *x*, contain somewhat more hemoglobin than most of the cells, and *xx* contrasts still more sharply with the surrounding cells by reason of a very deep pink color, the result of relatively more hemoglobin.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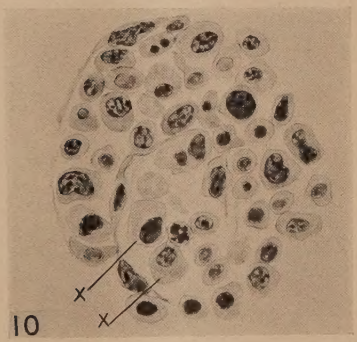
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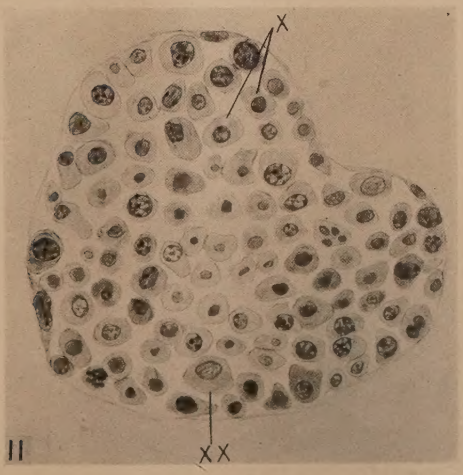
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